

Developing a Stratified Approach to Treatment in Type 2 Diabetes

Ealaff Y Shakweh

Masters by Research Thesis 2018

Developing a Stratified Approach to Treatment in Type 2 Diabetes

Volume 1 of 1

Submitted by Ealaff Shakweh to the University of Exeter as a thesis for the degree of *Masters by Research* in Medical Studies, 1 August 2018

This *thesis* is available for Library use on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.

I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

.....

Abstract

Type 2 diabetes (T2D) is a leading cause of global morbidity and mortality characterised by deficits in insulin secretion and function. Diabetes' poor health outcomes are largely attributed to its macrovascular and microvascular complications, which can be prevented by ensuring adequate glycaemic control. Current guidance recommends optimising lifestyle interventions (weight loss and exercise) as first line management in T2D progressing to oral pharmacotherapy with metformin. If however poor glycaemic control persists, second and third line agents are added. The numerous glucose-lowering agents available and a lack of robust comparative outcome data make selecting the most appropriate second and third line therapies for individuals with T2D a major clinical dilemma.

There is huge inter-individual variation in response to type 2 diabetes medication: some individuals may elicit a significant glucose-lowering response to one medication, but the same medication may have little effect on others. As diabetes is a heterogeneous disease (a disease likely manifesting from multiple aetiologies) it is likely that a biological basis partly underpins the variation in therapeutic response. These disease attributes suggest the potential for the integration of a stratified approach to diabetes therapy. Stratification involves prescribing medication based on factors other than patients' presenting symptoms; such as phenotypic traits or biomarkers. The main aim of stratification is to identify and provide individualised therapies that maximise therapeutic efficacy and minimise adverse events.

Limited research has been conducted into the mechanisms underpinning variation in diabetes treatment response. The main aim of this thesis is to investigate potential mechanisms underpinning the inter-individual variation in response to sitagliptin (a DPP-4 inhibitor) and gliclazide (a sulphonylurea) using data from the MASTERMIND randomised control crossover trial. This will aid in understanding variation in therapeutic response in T2D and support the development of a stratified approach.

Chapter 1 provides an overview of stratified medicine and introduces T2D as a potential candidate for stratification. In this chapter, we also review the literature

surrounding the pharmacokinetics of sitagliptin and gliclazide, the agents investigated in this thesis to answer questions of stratification and inter-individual variation in therapeutic response.

Chapter 2 provides a detailed overview of the MASTERMIND randomised control crossover study design, from which data for this thesis was analysed.

Chapter 3 explores potential associations between trough and total plasma drug levels and (fasting and post-prandial plasma) glucose response to sitagliptin and gliclazide. We found that while plasma drug levels were highly variable this variation did not substantially explain short term glycaemic response to these agents.

In Chapter 4 we assess whether medication adherence, calculated using medication possession ratio (MPR) is associated with therapeutic response to sitagliptin and gliclazide therapy. We found that, in a trial setting most participants were adherent to study medication and that variation in adherence did not explain variation in glucose-lowering response in this setting.

In Chapter 5 we explore whether measurable differences in lifestyle factors explain the variation in glucose-lowering response to sitagliptin and gliclazide by analysing weight and accelerometer data. We show that both weight and physical activity were stable within an individual during the study period and that individual differences of these lifestyle factors between each treatment period, do not explain the corresponding differences in glycaemic responses.

Chapter 6 investigates whether glucose-lowering response to diabetes therapy is specific to an individual. We show that the change in fasting glycaemia from after stopping sulphonylurea (SU) therapy strongly correlates with the fasting glycaemic response observed when re-starting SU therapy but does not predict fasting glycaemic response to DPP-4 inhibitor therapy.

An overview of the major finding of each chapter and their implications for future precision medicine research in diabetes is discussed in Chapter 7.

Table of Contents

Abstract	3
List of Figures	9
List of Tables	11
Acknowledgements	13
Abbreviations	14
1. Introduction	16
1.1 Applying a Stratified Approach to Type 2 Diabetes	17
1.1.1 The Burden of Type 2 Diabetes Mellitus	17
1.1.2 Current Treatment Pathways in Type 2 Diabetes	19
1.1.3 Response to glucose-lowering therapy is highly variable	19
1.1.4 Heterogeneity in the Pathogenesis of Type 2 Diabetes Mellitus	20
1.1.5 Applying a Stratified Approach to Type 2 Diabetes	21
1.1.5.1 Defining ‘Stratified Medicine’	21
1.1.5.2 Success Stories in Stratified Medicine	22
1.1.5.3 Is T2D is an appropriate condition for stratification?	24
1.2 The potential role of variation in drug levels for explaining variation in response.	26
1.2.1 Drug levels as a potential explanation for variation in therapeutic response	26
1.2.2 Overview of Sitagliptin	28
1.2.2.1 Mechanism of Action	28
1.2.2.2 Pharmacokinetics of sitagliptin	28
1.2.2.3 Dose-response studies of sitagliptin	30
1.2.3 Overview of Gliclazide	32
1.2.3.1 Mechanism of Action	32
1.2.3.2 Pharmacokinetic properties of Gliclazide	32
1.2.3.3 Dose- response studies of sulphonylureas	34
Aims	36
Objectives	36
1.3 References	37
Chapter 2: Data Source and Methods	44
Data Source: <i>The MASTERMIND Randomised Control Crossover Trial</i> (NCT01847144)	45
2.1 Study Procedures and Flow	45
2.1.1 Screening and Identification	45
Recruitment	45
Study Visits	48

2.2 Part One: Visit 1: Baseline	48
Treatment Cessation and Monitoring	48
Visit 2: Mixed Meal Tolerance Test (location: research centre)	48
Visit 3: Assessment of Glycaemia	48
2.3 Part 2: Randomisation and Adherence	49
Visit 4: Mixed Meal Tolerance Test (location: research centre)	49
Visit 5: Assessment of glycaemia and initiation of second study treatment	49
Visit 6: Mixed Meal Tolerance Test (location: research centre)	50
Visit 7: Assessment of Glycaemia	50
Randomisation	50
Concomitant Therapies	50
Pregnancy	50
Subject Withdrawal	52
Ethics and Regulatory Approvals	52
2.4 Understanding the study design	53
Crossover Design	53
Advantages of the crossover design	53
Washout period	53
Assessment of glycaemic response	54
2.5 References	55
Chapter 3	56
Chapter 3: Precision medicine in Type 2 Diabetes: is variation in response to sitagliptin and gliclazide therapy related to drug levels?	57
3.1 Introduction	57
3.2.1 Study Design	58
3.2.2 Recruitment	58
3.2.2 Study procedure	60
3.2.4 Randomisation	62
3.2.5 Biochemical analysis: Measurement of drug levels	62
3.2.6 Statistical analysis	62
3.3 Results	64
Participant characteristics	64
Pharmacokinetics	66
Glycaemic response to therapy	67
3.4 Discussion	74
3.5 Conclusion	78
3.6 Supplementary Tables	79

3.7 References.....	81
Chapter 4	83
Chapter 4: can variation in adherence explain the variation in glycaemic response to sitagliptin and gliclazide therapy?	84
4.1 Introduction	84
4.2 Methods.....	85
4.2.1 Study design.....	85
4.2.2 Measurement of Adherence	85
4.2.4 Statistical Analysis.....	86
4.3 Results.....	87
4.4 Discussion.....	92
4.5 Conclusion	95
4.6 References.....	96
Chapter 5	98
Chapter 5: measurable differences in weight and physical activity do not explain variation in response to sitagliptin or gliclazide therapy.	99
5.1 Introduction	99
5.2 Methods.....	100
Assessment of weight.....	100
Measurement and categorisation of physical activity	100
Statistical analysis	100
5.3.1 Results - <i>Weight</i>	102
5.3.2 Results - <i>Physical Activity</i>	108
5.4 Discussion.....	117
5.5 Conclusion	121
5.6 References.....	122
Chapter 6	124
Chapter 6: is response to glucose-lowering therapy specific to an individual?	125
6.1 Introduction.....	125
6.2 Methods.....	126
6.2.1 Study design.....	126
6.2.2 Statistical analysis	126
6.3 Results.....	127
6.4 Discussion.....	130
Comparison with other studies	130
Strengths and limitations	130
Clinical implications for future research	131
6.5 Conclusion	133

6.6 References.....	134
Chapter 7	135
Chapter 7: Overall Conclusions from Thesis	136
7.1 Summary.....	136
7.2 Strengths of our findings.....	136
7.3 Limitations of our findings.....	137
7.4 Clinical implications	138
7.5 Future research.....	139
7.6 Final Remarks	140
7.7 References.....	141

List of Figures

Figure 1- MASTERMIND consort diagram.....	47
Figure 2 - MASTERMIND Study overview- obtained directly from MASTERMIND Study Protocol.....	51
Figure 3- Mastermind CONSORT flow diagram	59
Figure 4 - <i>Study design. Participants withdrew sulphonylurea therapy for up to 2 weeks followed by an optional cross-over extension where they were randomized to 4 weeks of treatment with gliclazide (sulphonylurea) or sitagliptin (DPP-4 inhibitor), followed by 4 weeks of the second treatment with a 2 week wash-out period in between. *Study medication was administered 15 minutes after commencing MMTT.</i>	61
Figure 5- Line graph (with error bars representing the interquartile range) showing median plasma drug level of sitagliptin (a) and gliclazide (b) over time in on treatment mixed meal tolerance tests. Daily dose given immediately after time 0.....	66
Figure 6 - box and whisker plot demonstrating relationship between trough levels of gliclazide and eGFR (a) and box and whisker plot demonstrating how decreasing glomerular filtration rate (according to CKD classification) increases trough levels of sitagliptin (b).	68
Figure 7- scatter plots showing the association between: fasting glucose levels and trough plasma drug levels of sitagliptin (a) and .gliclazide (b) *all glucose outcomes have been adjusted to mean baseline glucose of 9.3mmol/L for fasting glucose outcomes and were also adjusted for drug order.	70
Figure 8- scatter plots showing the association between: incremental AUC _{0-240min} glucose and trough plasma drug levels of sitagliptin (a) and gliclazide (b),*all glucose outcomes have been adjusted to mean baseline glucose of 417.4 for incremental AUC _{0-240min} . All glucose outcomes were also adjusted for drug order.	71
Figure 9- scatter plots showing the association between incremental AUC _{0-240min} plasma glucose and total AUC _{0-240min} drug levels of sitagliptin (a) and gliclazide (b) *all glucose outcomes have been adjusted to mean baseline glucose of 9.3mmol/L for fasting glucose outcomes and 417.4 for incremental AUC _{0-240min} . All glucose outcomes were also adjusted for drug order.	73
Figure 10 Bar chart representing number of individuals who were adherent to sitagliptin (left) and gliclazide (right)	87
Figure 11 histograms representing the distribution of the change in weight from baseline on sitagliptin therapy (a) and gliclazide therapy (b)	102
Figure 12 scatter graph showing correlation between mean weight on sitagliptin (x axis) and mean weight on gliclazie (y axis)	103

Figure 13- scatter plots showing the association between: fasting glucose levels and change in weight from baseline on sitagliptin (a) and gliclazide (b) *all glucose outcomes have been adjusted to mean baseline glucose of 9.3mmol/L for fasting glucose outcomes and were also adjusted for drug order.	104
Figure 14 scatter plots showing the association between: incremental AUC _{0-240min} glucose levels and change in weight from baseline on sitagliptin (a) and gliclazide (b) *all glucose outcomes have been adjusted to mean baseline glucose of 414.7mmol/L for incremental AUC _{0-240min} outcomes and were also adjusted for drug order.	105
Figure 15- scatter graphs showing the association between light (a) and moderate-to-vigorous (b) physical activity during treatment period 1 and treatment period 2	109
Figure 16 : scatter plots showing the association between: light physical activity and fasting plasma glucose for sitagliptin (a) and gliclazide (b) and light physical activity and incremental AUC _{0-240min} for sitagliptin (c) and gliclazide (d). <i>*all glucose outcomes have been adjusted to mean baseline glucose of 9.3mmol/L for fasting glucose outcomes and 417.4 for incremental AUC_{0-240min}. All glucose outcomes were also adjusted for drug order.</i>	112
Figure 17- scatter plots showing the association between: moderate-to-vigorous physical activity and fasting plasma glucose for sitagliptin (a) and gliclazide (b) and moderate-to-vigorous physical activity and incremental AUC _{0-240min} for sitagliptin (c) and gliclazide (d). <i>*all glucose outcomes have been adjusted to mean baseline glucose of 9.3mmol/L for fasting glucose outcomes and 417.4 for incremental AUC_{0-240min}. All glucose outcomes were also adjusted for drug order.</i>	113
Figure 19- scatter graph showing a significant correlation within an individual between change in fasting glucose 1 week after withholding sulphonylurea therapy (x axis) and average change in fasting glucose on gliclazide therapy, administered per study protocol (y-axis).	127
Figure 20- scatter graph showing no evidence of a relationship between change in fasting glucose 1 week after withholding sulphonylurea therapy (x axis) and average change in fasting glucose on sitagliptin therapy within an individual (y-axis).	128
Figure 21- scatter graph showing no significant correlation between fasting glycaemic responses to gliclazide (x- axis) and sitagliptin therapy (y-axis).	129

List of Tables

Table 1- Inclusion/Exclusion criteria.....	46
Table 2- baseline characteristics of participants. Results expressed as median (IQR) unless specified otherwise.	65
Table 3- Association of trough drug levels of sitagliptin and gliclazide with clinical determinants, using univariate linear regression analysis.	69
Table 4- Association of total AUC ₀₋₂₄₀ drug levels of sitagliptin and gliclazide with clinical determinants, using univariate linear regression analysis.	69
Table 5- : analysis performed using ANCOVA to determine if individuals within the 25 th centile of trough drug levels have a difference in glycaemic response to glucose-lowering therapy compared to those with greater trough drug levels, adjusted for baseline glucose and drug order.	72
Table 6 - continuous association between adherence and glycaemic response (fasting, total AUC _{0-240min} , incremental AUC _{0-240min} and fructosamine. Analysis was performed using linear regression with adjustment for baseline glucose and drug order.	89
Table 7- analysis performed using ANCOVA to determine if individuals who were adherent have a difference in glycaemic response to glucose-lowering therapy compared to those who were not adherent. Results are shown at the means of baseline glucose and drug order.	91
Table 8- Association between change in weight from baseline on sitagliptin and gliclazide and therapeutic response (fasting, total AUC _{0-240min} , incremental AUC _{0-240min} , and fructosamine response. All glycaemic outcomes have been adjusted for baseline glucose and drug order.	106
Table 9- relationship between differences in weight on sitagliptin and gliclazide and the differences in glycaemic outcomes on sitagliptin and gliclazide. All linear regression outcomes have been adjusted for drug order.	107
Table 10 - baseline characteristics of participants with physical activity measurements. Results expressed as median (IQR) unless specified otherwise.	108
Table 11- <i>relationship between baseline clinical characteristics and physical activity (LPA and MVPA) using Spearman's correlation, with the exception for gender (a categorical variable) which was assessed using Mann-Whitney U.</i>	110
Table 12- continuous association between MVPA and glycaemic response to sitagliptin (above) and gliclazide (below). Analysis performed using linear regression with outcomes adjusted for baseline glucose and drug order.	114

Table 13- continuous association between LPA and glycaemic response to sitagliptin (above) and gliclazide (below). Analysis performed using linear regression with outcomes adjusted for baseline glucose and drug order.114

Table 14- results from linear regression analysis, showing no significant association between differences in glycaemic response between sitagliptin and gliclazide therapy and differences between light physical activity (top) and moderate-to-vigorous physical activity (bottom) between therapies. Glycaemic outcomes were adjusted for drug order.116

Acknowledgements

I am hugely grateful to my supervisors Dr Angus G Jones and Dr Beverley Shields for their guidance and mentorship over the last year. I would also like to thank Professor Andrew Hattersley for welcoming me into his research team and for demonstrating how robust research, collaboration and good luck can make a real difference to clinical practice.

I thank all members of Professor Hattersley's research team; particularly Dr Tim McDonald, Dr Richard Oram, Dr Kashyap Patel, Dr Andrew Wood and Dr Lauren Rodgers for teaching me invaluable research skills over the last year.

I would also thank my pastoral tutor Dr Kate Ellacott for her support and unchallenged belief in me over the past year; she is truly an asset to the University of Exeter.

Finally, I would like to thank my parents and my sister, Eathar to whom this thesis is dedicated.

Abbreviations

ANOVA	Analysis of variance
ADOPT	A Diabetes Outcome Progression Trial
ADVANCE Trial	Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation Trial
AUC	Area Under Curve
β	Regression coefficient, change in outcome variable for a 1 unit change in covariate
BMI	Body Mass Index
CPRD	Clinical Practice Research Datalink
CLCR	Creatinine Clearance rate
DPP-4	Dipeptidyl Peptidase- 4
eGFR	Estimated Glomerular Filtration Rate
FPG	Fasting Plasma Glucose
GCK	Glucokinase
GLP-1	Glucagon Like Peptide- 1
GLP1A	Glucagon Like Peptide -1 Agonist
GoDarts	Genetics of Diabetes and Audit Research Tayside Study
HbA1c	Haemoglobin A1c/ Glycosylated Haemoglobin
IQR	Interquartile Range
LPA	Light physical activity

MEMS Caps	Medication Event Monitoring System caps (the gold standard in measuring medication adherence)
MMTT	Mixed Meal Tolerance Test
MODY	Maturity Onset Diabetes of the Young
MVPA	Moderate-to-vigorous physical activity
NHS	National Health Service
NICE	National Institute for Health and Clinical Excellence
OD	Once Daily
PPG	Postprandial Glucose
SD	Standard Deviation
SU	Sulphonylurea
T2D	Type 2 Diabetes

1. Introduction

1.1 Applying a Stratified Approach to Type 2 Diabetes

1.1.1 The Burden of Type 2 Diabetes Mellitus

With an estimated global prevalence of 422 million [1], Type 2 Diabetes Mellitus (T2D) is a significant burden to modern society. This figure is expected to rise to 642 million by 2040 [1], and can largely be attributed to sedentary lifestyle, an ageing population and an increased incidence in obesity [2].

T2D is a disease characterised by deficits in insulin secretion and function, leading to raised blood glucose levels (hyperglycaemia) [3]. Observational studies have reported a relationship between the extent of hyperglycaemia and an increased risk of mortality and of macrovascular (cerebrovascular and cardiovascular) and microvascular (neuropathy, nephropathy and retinopathy) disease within T2D [4–6]. Unfortunately these complications cause significant health burdens, including; coronary heart disease, lower extremity amputations, end stage renal failure, and blindness [7].

The effectiveness of tight glycaemic control in preventing diabetes' microvascular complications was established by the Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) study for type 1 diabetes (T1D)[8, 9]. The United Kingdom Prospective Diabetes Study (UKPDS) reported how optimising glycaemic control also protected from microvascular complications in T2D and demonstrated a reduction in macrovascular disease in long term follow up [10].

The annual cost of treating diabetes and its complications to the National Health Service (NHS) in 2016 was reportedly £14 billion[11], with the International Diabetes Federation (IDF) reporting 12% of the global health expenditure spent on managing the condition [12]. Despite these huge expenses, robust evidence linking sustained hyperglycaemia to poor health outcomes and the numerous glucose-lowering agents on the market, glycaemic control remains sub-optimal in many patients with T2D [13]. The following section will review current treatment

pathways and the challenges faced in pursuit of effective glycaemic management.

1.1.2 Current Treatment Pathways in Type 2 Diabetes

Current guidance from the National Institute for Health and Care Excellence (NICE) [14], recommends optimising lifestyle interventions as first line in the management of T2D. If blood glucose levels remain poorly controlled, oral pharmacotherapy is commenced, typically with metformin with new agents added (and rarely withdrawn) in a stepwise fashion. There are several hypoglycaemic medications available, with the most common current second-line agents prescribed in the UK being DPP-4 inhibitor and/or sulphonylurea therapy[15]. If patients fail to achieve adequate glycaemic control on oral therapy, eventually insulin injections are required [16].

The lack of robust comparative effectiveness data make selecting second and third-line treatment intensifications a huge clinical dilemma, as evidenced by recent guidelines[17, 18]. Due to this, choice of second-line glucose-lowering agents is largely dictated by cost, physician bias and side effect profile, (rather than choosing the most effective drug for that individual).

1.1.3 Response to glucose-lowering therapy is highly variable

In T2D there is huge variation seen in response to glucose-lowering therapy [18–21]; some individuals elicit a marked glucose-lowering response to one drug, whilst other individuals exhibit no response to the same drug. The reasons underpinning the variation in therapeutic response remain unclear.

There are several possibilities for heterogeneity in response to glucose lowering therapy including; pharmacokinetic differences (i.e. differences in drug absorption, transport or metabolism) and pharmacodynamics differences (variation in responses occurring when the drug is at the site of action). As diabetes drugs target pathways that are defective, it is possible that that the wide variation in therapeutic response may reflect the aetiologies underpinning the disease[19].

1.1.4 Heterogeneity in the Pathogenesis of Type 2 Diabetes Mellitus

Diabetes is a heterogeneous disease, best described by Stephen Fajans in his 1978 Banting Lecture as a syndrome *“comprised of a variety of diseases all characterised by hyperglycaemia and tissue changes that result from heterogeneous aetiological and pathogenetic factors”* [20]. These factors include an ageing population and associated transitions in lifestyle and culture (such as reduced physical activity and an increased incidence in obesity).

The variation in insulin resistance and deficiency within the T2D cohort may reflect the existence of phenotypically heterogeneous subgroups with specific pathophysiological characteristics [21]. Furthermore, there is evidence to suggest that the variation in T2D pathogenesis and its precursor states may be influenced by biological factors such as age, gender and ethnicity [22]. It is possible that subgroups of patients with similar biological characteristics and phenotypes, have similar therapeutic responses to medications. By investigating glucose homeostasis and therapeutic response amongst and between these subgroups, it may be possible to identify predictors of therapeutic response, allowing for a stratified approach to the medical management of diabetes.

1.1.5 Applying a Stratified Approach to Type 2 Diabetes

1.1.5.1 Defining 'Stratified Medicine'

The heterogeneity in T2D and in the therapeutic response to glucose-lowering agents suggests that diabetes may be an ideal condition for a stratification. Stratified medicine is defined as “the differential prescribing of medications or treatment regimens to groups of individuals based on attributes other than their presenting symptoms resulting from their disease” [23]. In essence, stratification is a form of medicine that categorises a population into the most appropriate biological groups to determine the optimal therapeutic response [24].

Stratified medicine is a term often used interchangeably with 'precision' or 'personalised' medicine; however some authors argue subtle differences [25] [26]. Precision medicine promotes the finer sub-classification of disease, by including repeated monitoring of biomarkers to enable the re-tailoring of treatment according to an individual's response [25]. Personalised medicine however, incorporates a precise biological stratified approach to treatment in addition to appreciating holistic needs of the patient (by encouraging patient participation and preference) [26]. By identifying therapies that are most effective for an individual or a subgroup of patients, stratified medicine serves to maximise therapeutic efficacy and minimise adverse medication effects, thus improving clinical care.

NHS England has promoted a 'personalised medicine' approach in their NHS England 2016 strategy, recognising the importance of both biological and social influences in healthcare [25]. This government initiative, highlights how the development and implementation of stratification represents a new era in medicine; replacing the 'one size fits all' and 'trial and error' approach that permeates current practice [25].

1.1.5.2 Success Stories in Stratified Medicine

The recognition of breast cancer as a heterogeneous disease and subsequent implementation of a stratified approach has revolutionised its treatment and has significantly reduced mortality rates [27]. In 2012, Curtis and colleagues reported over 10 subgroups of breast malignancy and confirmed the disease is heterogeneous at a genetic level; with each subgroup responsive to a different treatment [28]. Categorising individuals into subgroups necessitates the histological grading of the tumour, assessment of hormonal receptor expression and the amplification status of the HER2 gene. The use of HER2 as a biomarker has become a paramount diagnostic tool and intrinsic to dictating treatment. If the HER2 gene is amplified, as in 15% of breast malignancies [29], individuals are treated with the monoclonal antibody trastuzumab. This treatment regime has dramatically improved patient outcomes; increasing 10-year disease-free survival rates from 62.2% to 73.7% [27]. Conversely, if histological analysis confirms the expression of oestrogen receptors, the oestrogen receptor antagonist tamoxifen is prescribed (or in post-menopausal women, the aromatase inhibitor anastrozole) due to its increased therapeutic efficacy [30].

Diabetes has also seen the benefits of stratification following the identification of monogenic subgroups facilitating tailored treatment regimens. Maturity Onset Diabetes of the Young (MODY) is a form of monogenic diabetes; diabetes that is caused by a single gene mutation, with the most common being mutations in the transcription factor gene HNF1A [31]. MODY comprises a group of autosomal dominant disorders and accounts for 1-2% of all cases of diabetes, with patients being diagnosed typically before the age of 25 [24]. Due to its non-ketotic and/or non-acute presentation, MODY was once confused with cases of T2D in children and adolescents [32]. Despite this, patients were and are still frequently misdiagnosed with having T1D due to their young presentation.

To date, pathogenic variants of 14 genes have been identified to cause MODY, with the most common being: GCK-MODY, and hepatic nuclear factor (HNF) 1A-, 4A- and 1B- MODY. Each specific MODY genetic subtype has a unique clinical picture and determines a specific treatment regimen [24]. Patients with GCK-MODY have stable, raised plasma glucose levels, however patients with transcription factor-linked MODY show a progressive deterioration of plasma glucose levels over time [24]. Patients with the HNF4A mutation present with

foetal macrosomia and neonatal hypoglycaemia, whilst glycosuria is a more typical symptom of individuals with HNF1A-MODY. In patients with HNF1B MODY however, organ- related developmental disorders predominate [33].

The most significant clinical feature associated with precision medicine in patients with MODY is the differential treatment response in its genetic subgroups [24]. As previously mentioned, MODY patients were often misdiagnosed with T1D and therefore treated with insulin. However, patients with GCK-MODY were observed to have similar average blood glucose levels irrespective of whether they receive insulin, sulphonylurea therapy, or no glucose lowering therapy at all [34]. This finding has meant that , most patients with GCK-MODY do not require glucose-lowering therapy [33–36]. Similarly individuals with *HNF1A*- and *HNF4A*- MODY exhibit high sensitivity to sulphonylureas [33, 35–38]. Stratified medicine has revolutionised therapy for these patients with MODY; as following the confirmation of their genetic diagnosis, insulin injections can be safely discontinued.

1.1.5.3 Is T2D is an appropriate condition for stratification?

The heterogeneity in the pathogenesis and treatment response in T2D suggests that the condition may benefit from stratification. It is possible that subgroups of patients with similar biological characteristics and phenotypes, have similar responses to glucose-lowering agents. By investigating glucose homeostasis and therapeutic response amongst and between these subgroups, it may be possible to identify predictors of therapeutic response and identify patients that are most likely to respond to therapy and/or least likely to suffer adverse effects [39]. This will prevent the unnecessary expense of ineffective treatments whilst also ensuring maximal therapeutic benefit and minimising harm. Unfortunately, very little is known about the best approach to studying variation of treatment response in diabetes or in other chronic diseases and key questions remain unanswered.

Is variation in response a reproducible biological characteristic of an individual which can therefore potentially be predicted?

Blood glucose levels fluctuate daily, however the extent to which glycaemic response to medication is influenced by reproducible and predictable intrinsic factors as opposed to extrinsic factors (such as diet, physical activity and medication adherence) is unknown.

Variations in extrinsic factors contribute to 'noise' when assessing variations in glycaemia in clinical practice and in research cohorts. Understanding the contribution of these factors may aid design of studies in this area, as measuring (and adjusting for) these factors will provide a more robust and accurate assessment glycaemic variation. If variation in apparent glucose lowering response is entirely due to 'noise' and not a biological characteristic of an individual, then a stratified approach to T2D will not be feasible.

Is variation in response to glucose-lowering therapy specific?

Although some individuals appear to be non-responders to hypoglycaemic agents, the extent to which the lack of response in clinical practice reflects non-adherence rather than an intrinsic lack of response is yet to be determined. Furthermore, it may be that intrinsic poor response to therapy is an attribute of an individual and does not vary by agent, in other words a patient may have disease features that mean they respond poorly to all medication. A stratified approach to diabetes therapy will only be possible if robust clinical trials demonstrate that poor

response to one medicine may be associated with good response to another glucose-lowering agent with a different mechanism of action; rather than a lack of response to all categories of glucose lowering medication.

1.2 The potential role of variation in drug levels for explaining variation in response.

1.2.1 Drug levels as a potential explanation for variation in therapeutic response

A potential candidate that may influence variation in treatment response is variation in achieved plasma drug levels. For most hypoglycaemic agents in T2D, it is standard practice to prescribe a standard/single dose of a drug, irrespective of an individual's characteristic (such as body weight, height etc). It is therefore likely that different individuals have different exposure to a drug; a potential cause of variation therapeutic response. Plasma drug concentrations have been shown to be associated with therapeutic response to treatment in several other diseases [40–42]; however whether variations in plasma drug levels are associated with variation in glycaemic response in T2D is not known.

Outside of diabetes studies, research in a number of conditions have shown relationships between drug levels and response, although these studies are often limited by small sample size [40–42]. Studies performed in patients with rheumatoid arthritis (RA) have shown evidence that drug levels of biological agents, such as adalimumab (ADA), are related to treatment response[41, 43–45]. One study investigated the association between serum ADA concentrations, anti-ADA antibodies and therapeutic response in 121 patients with RA treated with adalimumab over a 28 week period [43]. Response was assessed using the European League Against Rheumatism (EULAR) criteria and the change in Disease Activity Score 28 (DAS-28) score, with serum samples collected at baseline, 4, 16 and 28 weeks. Its results showed that patients with anti-ADA antibodies present had lower drug levels of adalimumab and showed a significantly poorer response to compared to those without antibodies detected (median(range) was 1.2 mg/l(0.0-5.6) vs 11.0 mg/l (2.0-33.0) respectively; $p < 0.001$) [43]. Results from this study also showed that good-responders had significantly higher plasma drug levels of adalimumab compared to non-responders ($p = 0.001$) [43].

There is also evidence within the literature to suggest an association between plasma concentrations of certain cancer drugs and therapeutic response [40]. A recent study assessed whether low plasma trough levels of the BCR-ABL tyrosine kinase inhibitor, imatinib were associated with a failure to complete a cytogenic response (CCR) or a major molecular response (MMR) in patients diagnosed with Chronic Myelogenous Leukaemia (CML) [40]. Patients were either treated with 400mg once daily (OD) (n=50) or 600mg OD (n=18). Results from this study showed that trough imatinib concentrations were significantly higher in patients who elicited a complete cytogenic response (n=56; p=0.03) and a major molecular response (n=34; p<0.001) compared to those who failed to elicit either response [40].

Results from the same study also revealed that plasma trough drug levels were highly variable amongst participants in both groups; ranging from 181 to 2947 ng/mL. Mean trough imatinib levels and their standard deviations (SD) were 1058 +/-557 ng/mL and 1444 +/-710 ng/mL for the 400-mg and 600-mg daily dose regimen respectively [40]. Multiple factors may have influenced the inter-individual variability of in plasma drug levels, including; environmental factors, genetic polymorphisms, co-administered drugs and concomitant illness. The wide variation in plasma drug concentrations highlights the importance of understanding the pharmacokinetics of the drug and biological factors that may implicate them. This is of particular importance when plasma drug levels are associated with therapeutic response or adverse effects.

There is very little evidence in the literature reporting on the relationship between plasma levels of glucose-lowering medications and therapeutic response. Thus, investigating this relationship is the logical first step in identifying mechanisms underpinning inter-individual variation in response to diabetes therapy. Achieved plasma drug levels depend on their pharmacokinetics and biological factors that influence them. The pharmacokinetics of the two of the most commonly prescribed agents prescribed after metformin, sitagliptin and gliclazide [15], are described below.

1.2.2 Overview of Sitagliptin

1.2.2.1 Mechanism of Action

Sitagliptin is a potent inhibitor of the dipeptidyl peptidase – 4 (DPP-4) enzyme, an enzyme degrades endogenous incretins. Incretins, such as glucagon like peptide-1 (GLP-1), are insulinotropic hormones released from the L-cells of the small intestine that stimulate approximately 70% of postprandial glucose-dependent insulin secretion. In the fasted state, incretins circulate at basal levels, however they rapidly increase in response to meals, in order to control variations in glycaemia[46–48]. Glycaemic regulation is achieved via multiple mechanisms, including: increased insulin secretion, decreased glucagon secretion, delayed gastric emptying and increased satiety [46, 48, 49].

1.2.2.2 Pharmacokinetics of sitagliptin

Absorption

Following the administration of a single dose of sitagliptin 100mg, pharmacokinetic studies performed on healthy volunteers report mean maximum plasma concentrations (C_{max}) of 950 nmol/L (after a median of 1-4 hours) [50, 51]. The mean area under the time curve (AUC) plasma concentration was 8.52 ng/mL·h [51]. Sitagliptin has a mean absolute bioavailability of 87% and its oral absorption is not implicated by food (therefore can be taken independently of meals). In healthy volunteers, single oral doses of sitagliptin 25-400mg resulted in the AUC plasma concentrations increasing in a dose dependent manner [50].

Distribution

Following the single 100mg intravenous administration of sitagliptin in healthy volunteers, the volume distribution at a steady state was reported to be 198L[51, 52]. The fraction of sitagliptin that reversibly binds to plasma protein is low at 38%. As it is the unbound fraction which exhibits pharmacologic effects, the potential for drug-drug interactions by plasma protein binding displacement is low. The equilibrium blood-to-plasma concentration ratio of sitagliptin is 1.21 [51, 52].

Metabolism and elimination

Metabolism serves a minor role in the elimination of sitagliptin, as approximately 80% of the administered dose remains unchanged when excreted in the

urine[52]. Vicent and colleagues demonstrated how following the oral administration of a single radiolabelled dose of sitagliptin, the 87% of sitagliptin was excreted unchanged in the urine [53]. It is then excreted through glomerular filtration and secretion into tubules. The remainder of the drug undergoes hepatic metabolism via isoenzymes CYP3A4 and CYP2C8 and excreted in the faeces[53–55]. The terminal elimination half-life of sitagliptin is 12.4 hours and the renal clearance is approximately 350 mL/min[51, 55].

Results from in–vitro studies have shown that sitagliptin does not influence cytochrome p450 enzyme activity [51, 55]. In individuals with normal renal function CYP3A4 (a member of the cytochrome p450 family) minimally contributes to the clearance of sitagliptin, however in patients with severe renal impairment, this isoenzyme may have a more influential role. As a result of this, potent inhibitors of CYP3A4 (including commonly prescribed drugs clarithromycin, ketoconazole and ritonavir) may influence the pharmacokinetics of sitagliptin, however this is yet to be evidenced by clinical trials. Potential pharmacokinetic interactions of commonly prescribed drugs in the T2D cohort have also been assessed [51, 55], clinically significant pharmacokinetic changes were seen when sitagliptin was co-administered with: sulphonylureas, metformin, simvastatin, digoxin, warfarin and oral contraceptives.

Influence of biological factors on pharmacokinetics

The pharmacokinetic profile of sitagliptin is similar in the type 2 diabetes cohort to that observed healthy individuals [51, 55]. Data obtained from phase I and II pharmacokinetic studies implied that no dosage adjustments are required for sitagliptin on the basis of age, race, body mass index and gender[51, 55]. Additionally, there were no clinically significant changes in exposure to sitagliptin in individuals with moderate hepatic impairment (Child-Pugh score 7-9) [54]. This is consistent with the limited hepatic metabolism of sitagliptin, thus dosage adjustments in this patient population is unnecessary. Contrastingly, guidance recommends adjusting sitagliptin dosages for patients with chronic kidney disease. The normal prescribed dose of sitagliptin is 100mg OD, however in patients with moderate renal impairment (GFR ≥ 30 to < 50 ml/min)., guidance recommends reducing the dose to 50mg OD. In patients with severe renal impairment (GFR < 30 ml/min) or end-stage renal disease (ESRD) requiring

dialysis, the dose should be reduced further to 25 mg OD. Following the oral administration sitagliptin 50mg, plasma AUC concentrations of sitagliptin increased twofold and fourfold in these populations respectively when compared to healthy volunteers [56] (increasing the risk of unwanted side effects such as; nasopharyngitis, upper respiratory tract infections and headaches) [57].

1.2.2.3 Dose-response studies of sitagliptin

A number of studies have reported the dose-response relationship for sitagliptin. A recent double-blinded single-dosed randomised control trial of sitagliptin evaluated the relationship between plasma DPP-4 activity and protection of intravenously infused GLP-1 [58]. Patients with T2D were administered different doses of sitagliptin: 0, 25, 100 and 200mg respectively. Results from this study revealed that sitagliptin inhibited DPP-4 activity in a dose-dependent manner from 25mg to 100mg (the latter being the current recommended clinical dose in individuals without renal impairment) [58]. Interestingly, there was no further effect on DPP-4 activity with the 200mg dose when compared with 100mg [58].

These findings are consistent with others reported in the literature. A previous double blinded, randomised control crossover trial conducted by Alba et al. [59] investigated whether a once daily regimen of sitagliptin 200mg was more effective at improving glycaemia (assessed by mean weighted glucose (MWG) over 24 hours) when compared to sitagliptin 100mg OD. This study also assessed and compared differences in DPP-4 enzyme inhibition activity between the doses [59]. Data from this study revealed that there was no significant difference observed in the 24 hour MWG between 100 and 200mg sitagliptin. This finding suggests that a 100mg dose of sitagliptin provides maximal glucose lowering efficacy. DPP-4 inhibition percentages were 80% and 85% (uncorrected) and 96% and 97% for 100mg and 200mg (corrected) of sitagliptin respectively [60].

One randomised, double-blinded, placebo control trial investigated the dose-ranging efficacy of sitagliptin in Japanese individuals with inadequate glycaemic control. In this study, 363 patients were randomised to one of the following arms: placebo, 25, 50, 100 or 200mg sitagliptin OD for 12 weeks [61]. Results showed no significant differences in HbA1c, FPG, post prandial glucose and glucose

AUC_{0-2h} concentrations between sitagliptin doses of 50- 100- and 200mg. The 25mg dose produced significantly smaller reductions in all of these parameters when compared to the 100 and 200mg doses [61].

In contrast to the above studies that suggest an optimal sitagliptin dose of 100mg, the study performed in Japanese individuals [61] showed no evidence of increased therapeutic efficacy with doses above 50mg. It is possible this difference may relate to differences in population characteristics, such as body weight or drug metabolism. Thus, understanding the influence of these clinical characteristics on the optimal therapeutic doses and efficacy sitagliptin is an avenue for further research.

.

1.2.3 Overview of Gliclazide

1.2.3.1 Mechanism of Action

Sulphonylureas are classified as insulin secretagogues as they stimulate insulin secretion from pancreatic beta cells. Consequently, their adverse effects are manifestations of increased insulin concentrations; including hypoglycaemia and weight gain[58, 62].

Sulphonylureas exert their hypoglycaemic effect by binding to the sulphonylurea receptor (SUR)-1 subunit of pancreatic B-cell ATP-sensitive potassium channels. This closes the potassium channels, increasing the concentration of intracellular potassium, which causes the beta cell membrane to depolarise. As a result of this, voltage-dependent calcium channels open, increasing intracellular calcium concentrations, causing the release of insulin via exocytosis [62].

1.2.3.2 Pharmacokinetic properties of Gliclazide

Absorption

The oral absorption of gliclazide is reported to be similar in healthy individuals and in patients with T2D [63]; with both populations exhibiting huge inter-individual variability in their pharmacokinetic parameters. Following the oral administration of gliclazide 40-120mg pharmacokinetic studies have reported the average time to achieve maximum plasma concentrations (T_{max}) as 2-8 hours, with combined literature reporting maximum plasma concentrations (C_{max}) as 2.2 µg/ml 8.0 µg/ml [63–66]. Both C_{max} and T_{max} reportedly increase in a dose dependent manner, with steady state (trough) plasma concentrations of gliclazide achieved after 2 days [65]. The rate of intestinal absorption of gliclazide has been shown to be highly variable due to its premature dissolution in the digestive system. Furthermore, there is evidence to suggest that agents which increase the solubility of gliclazide also increase plasma gliclazide levels [67].

Although absorption rates of gliclazide are highly variable, it is unclear how clinical features may implicate its pharmacokinetics due to limited studies. One small study has suggested that age influences the absorption parameters of gliclazide. Forette et al. compared differences in the pharmacokinetics of

gliclazide amongst a group of young women (n=5) mean age = 26 and a group of elderly women (n=5) mean age = 77 [68]. Results revealed that C_{max} values were significantly lower and the time to achieve these values were significantly longer in the elderly female group compared to the younger group. [68]. Unfortunately, this study is limited by its small sample size, challenging its statistical power and increasing the likelihood that these results are due to chance, therefore further analysis must be conducted in a larger data set to validate these findings.

Distribution

Gliclazide is largely distributed in extracellular fluid, with high concentrations found in the liver, kidneys, skin, lungs and skeletal muscle. Gliclazide has a low volume distribution of 13L to 24L (or 36.3% of bodyweight) in both healthy individuals and those diagnosed with T2D [63, 64], with reports of its volume distribution increasing with age [68]. Gliclazide has a high protein binding affinity (85%-97%), increasing the potential for drug-drug interactions by plasma protein binding displacement.

Metabolism and elimination

Gliclazide undergoes extensive hepatic metabolism by CYP450 enzymes. Gliclazide metabolites include oxidised and hydroxylated derivatives in addition to glucuronic acid conjugates. These metabolites are excreted in the urine via the kidneys (of which less than 1% of the drug remains un-metabolised). The reported elimination half-life of gliclazide is 8.1 hours to 20.5 hours and has a plasma clearance rate of gliclazide is 0.78 L/h (13 ml/min). Ings and colleagues reported that approximately 70% of the administered dose is excreted in the urine, reaching peak elimination rates 7 to 10 hours after administration [69, 70]. Metabolites can be detected in the urine 120 hours post administration. Faecal elimination, plays a minor role, accounting for approximately 11 % of the administered dose [64].

1.2.3.3 Dose- response studies of sulphonylureas

There is data to suggest a linear relationship between sulphonylurea dosages and its pharmacological effects at the low end of the dose range [71, 72]. The recommended maximum doses for glipizide and glibenclamide/glyburide is 40mg/day and 20mg/day respectively, however there is clinical evidence to indicate that maximal therapeutic effect of all of these sulphonylureas occurs at a dose of approximately 10mg/day, with no greater glycaemic control achieved at higher doses [71]. Analysis of data from UKPDS showed that in diet treated patients with a baseline fasting glycaemia of >10mmol/L maximum doses of sulphonylurea rarely achieve adequate fasting glycaemia (<6mmol/l) [10]. Some authors have reported that the only therapeutic benefit of increasing the dose of sulphonylureas was a slight prolongation of a glucose-lowering effect [72].

In support of these findings, a dose-response study of glimepride, conducted in 308 patients with Type 2 Diabetes showed that in participants who were given once daily doses of 1mg, 4mg and 8mg, there was no significant difference in glycaemic responses those who received 4mg and 8mg doses, evidenced by the flattening of a dose-response curve[73]. Furthermore, data obtained from two randomised placebo-controlled trials investigating the dose response characteristics of glipizide highlighted found no difference in the reduction of fasting, HbA1c and post prandial glycaemic response between six doses of glipizide (ranging from 5mg to 60mg once daily). However, further pharmacodynamic analysis revealed that that 5mg, 10mg and 20mg doses were the most effective in achieving maximum reductions in HbA1c and FPG respectively [74].

In contrast to the above findings, one placebo- controlled, double-blinded crossover trial suggested that the dose-response curve for sulphonylureas may be bell-shaped; with decreased glucose-lowering response at the higher end of the recommended dose range. This study investigated the therapeutic benefits of increasing the dosages of glipizide over a 9 month period in patients with T2D (n=23) [75]. Patients were randomised into 3 groups and received glipizide doses of 10, 20 and 40mg of glipizide for a 3 month period in random order. Results revealed that following a test meal, plasma glipizide concentrations increased

with increasing doses, however there was no significant association with increasing the dose and glycaemic response (assessed by HbA1c testing and home glucose monitoring) [75]. Results also showed a significantly lower insulin response to the test meal following the 40mg/day glipizide regimen compared to the 10mg/day regimen ($p=0.02$) [75]. Thus, the study concluded that there may be no therapeutic benefit to increasing the dose of glipizide above 10mg/day with an increased dose potentially reducing beta cell function [75].

While a number of studies have examined dose-response relationships between sitagliptin and gliclazide, literature investigating the association between achieved drug levels of glucose-lowering agents and response is scarce; thus highlighting a need for further research.

Aims

This Masters by Research aims to investigate potential mechanisms underpinning the variation in glucose response seen to sitagliptin and gliclazide. Results from this analysis will aid in determining whether a stratified approach can be applied to Type 2 diabetes therapy in addition to potential predictors of therapeutic response. This research will be performed using data collated from the MASTERMIND randomised control crossover pilot study (clinical trial identifier: (NCT01847144)).

Objectives

1. To determine whether differences in plasma drug levels explain variation in glucose-lowering response to DPP-4 inhibitor and sulphonylurea therapy.
2. To determine whether variation in drug response is a biological attribute of an individual.
 - a. To determine the degree to which non-concordance accounts for non-response to diabetes treatment in Type 2 Diabetes.
 - b. To determine whether lifestyle factors, specifically measurable changes in weight and physical activity, is a major component of variation in response to therapy.
3. To determine whether lack of response to one class of drugs is also associated with lack of response to an alternative agent with a different mechanism of action.

1.3 References

1. Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants - The Lancet. [https://www.thelancet.com/journals/lancet/article/PIIS0140-6736\(16\)00618-8/abstract](https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(16)00618-8/abstract). Accessed 18 May 2018
2. Zimmet PZ, Magliano DJ, Herman WH, Shaw JE (2014) Diabetes: a 21st century challenge. *The Lancet Diabetes & Endocrinology* 2:56–64 . doi: 10.1016/S2213-8587(13)70112-8
3. Meigs JB, Muller DC, Nathan DM, et al (2003) The natural history of progression from normal glucose tolerance to type 2 diabetes in the Baltimore Longitudinal Study of Aging. *Diabetes* 52:1475–1484
4. Stratton IM, Adler AI, Neil HA, et al (2000) Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ* 321:405–412
5. Khaw K-T, Wareham N (2006) Glycated hemoglobin as a marker of cardiovascular risk. *Curr Opin Lipidol* 17:637–643 . doi: 10.1097/MOL.0b013e3280106b95
6. Selvin E, Ning Y, Steffes MW, et al (2011) Glycated Hemoglobin and the Risk of Kidney Disease and Retinopathy in Adults With and Without Diabetes. *Diabetes* 60:298–305 . doi: 10.2337/db10-1198
7. Gregg EW, Sattar N, Ali MK (2016) The changing face of diabetes complications. *Lancet Diabetes Endocrinol* 4:537–547 . doi: 10.1016/S2213-8587(16)30010-9
8. Diabetes Control and Complications Trial Research Group, Nathan DM, Genuth S, et al (1993) The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977–986 . doi: 10.1056/NEJM199309303291401
9. Nathan DM, Cleary PA, Backlund J-YC, et al (2005) Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *N Engl J Med* 353:2643–2653 . doi: 10.1056/NEJMoa052187
10. (1995) United Kingdom Prospective Diabetes Study (UKPDS). 13: Relative efficacy of randomly allocated diet, sulphonylurea, insulin, or metformin in patients with newly diagnosed non-insulin dependent diabetes followed for three years. *BMJ* 310:83–88

11. Hex N, Bartlett C, Wright D, et al (2012) Estimating the current and future costs of Type 1 and Type 2 diabetes in the UK, including direct health costs and indirect societal and productivity costs: Estimating current and future costs of Type 1 and Type 2 diabetes in the UK. *Diabetic Medicine* 29:855–862 . doi: 10.1111/j.1464-5491.2012.03698.x
12. International Diabetes Federation. (2016) International Diabetes Federation. IDF Diabetes Atlas 7th edn. In: International Diabetes Federation. IDF Diabetes Atlas 7th edn. <http://www.diabetesatlas.org>
13. Blonde L, Aschner P, Bailey C, et al (2017) Gaps and barriers in the control of blood glucose in people with type 2 diabetes. *Diab Vasc Dis Res* 14:172–183 . doi: 10.1177/1479164116679775
14. Type 2 diabetes in adults: management | Guidance and guidelines | NICE. <https://www.nice.org.uk/guidance/ng28>. Accessed 22 May 2018
15. Curtis HJ, Dennis JM, Shields BM, et al Time Trends and Geographical Variation in Prescribing of Drugs for Diabetes in England 1998-2017. *Diabetes, Obesity and Metabolism*. doi: 10.1111/dom.13346
16. Excess Mortality among Persons with Type 2 Diabetes | NEJM. <https://www.nejm.org/doi/full/10.1056/NEJMoa1504347>. Accessed 18 May 2018
17. Inzucchi SE, Bergenstal RM, Buse JB, et al (2015) Management of Hyperglycemia in Type 2 Diabetes, 2015: A Patient-Centered Approach: Update to a Position Statement of the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care* 38:140–149 . doi: 10.2337/dc14-2441
18. McGuire H, Longson D, Adler A, et al (2016) Management of type 2 diabetes in adults: summary of updated NICE guidance. *BMJ* 353:i1575
19. Pearson E (2014) *RD Lawrence Lecture 2013* Stratified approaches to the management of diabetes. *Diabetic Medicine* 31:393–398 . doi: 10.1111/dme.12391
20. Fajans SS, Cloutier MC, Crowther RL (1978) The Banting Memorial Lecture 1978. Clinical and etiologic heterogeneity of idiopathic diabetes mellitus. *Diabetes* 27:1112–1125
21. Faerch K, Hulmán A, Solomon TPJ (2016) Heterogeneity of Pre-diabetes and Type 2 Diabetes: Implications for Prediction, Prevention and Treatment Responsiveness. *Curr Diabetes Rev* 12:30–41
22. Carter JS, Pugh JA, Monterrosa A (1996) Non-insulin-dependent diabetes mellitus in minorities in the United States. *Ann Intern Med* 125:221–232

23. Stratified medicine | The Academy of Medical Sciences. <https://acmedsci.ac.uk/policy/policy-projects/Stratified-medicine>. Accessed 18 May 2018
24. Hattersley AT, Patel KA (2017) Precision diabetes: learning from monogenic diabetes. *Diabetologia* 60:769–777 . doi: 10.1007/s00125-017-4226-2
25. Day S, Coombes RC, McGrath-Lone L, et al (2017) Stratified, precision or personalised medicine? Cancer services in the ‘real world’ of a London hospital. *Sociology of Health & Illness* 39:143–158 . doi: 10.1111/1467-9566.12457
26. Cribb A, Owens J (2010) Whatever suits you: unpicking personalization for the NHS. *J Eval Clin Pract* 16:310–314 . doi: 10.1111/j.1365-2753.2010.01390.x
27. Perez EA, Romond EH, Suman VJ, et al (2014) Trastuzumab plus adjuvant chemotherapy for human epidermal growth factor receptor 2-positive breast cancer: planned joint analysis of overall survival from NSABP B-31 and NCCTG N9831. *J Clin Oncol* 32:3744–3752 . doi: 10.1200/JCO.2014.55.5730
28. Curtis C, Shah SP, Chin S-F, et al (2012) The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature* 486:346–352 . doi: 10.1038/nature10983
29. Yeo B, Turner NC, Jones A (2014) An update on the medical management of breast cancer. *BMJ* 348:g3608
30. Cuzick J, Sestak I, Baum M, et al (2010) Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 10-year analysis of the ATAC trial. *Lancet Oncol* 11:1135–1141 . doi: 10.1016/S1470-2045(10)70257-6
31. Shepherd M, Shields B, Hammersley S, et al (2016) Systematic Population Screening, Using Biomarkers and Genetic Testing, Identifies 2.5% of the U.K. Pediatric Diabetes Population With Monogenic Diabetes. *Diabetes Care* 39:1879–1888 . doi: 10.2337/dc16-0645
32. Bonfig W, Hermanns S, Warncke K, et al (2011) GCK-MODY (MODY 2) Caused by a Novel p.Phe330Ser Mutation. *ISRN Pediatrics* 2011:1–5 . doi: 10.5402/2011/676549
33. Murphy R, Ellard S, Hattersley AT (2008) Clinical implications of a molecular genetic classification of monogenic beta-cell diabetes. *Nat Clin Pract Endocrinol Metab* 4:200–213 . doi: 10.1038/ncpendmet0778

34. Steele AM, Shields BM, Wensley KJ, et al (2014) Prevalence of vascular complications among patients with glucokinase mutations and prolonged, mild hyperglycemia. *JAMA* 311:279–286 . doi: 10.1001/jama.2013.283980
35. Anik A, Çatlı G, Abacı A, Böber E (2015) Maturity-onset diabetes of the young (MODY): an update. *J Pediatr Endocrinol Metab* 28:251–263 . doi: 10.1515/jpem-2014-0384
36. Shepherd M, Shields B, Ellard S, et al (2009) A genetic diagnosis of HNF1A diabetes alters treatment and improves glycaemic control in the majority of insulin-treated patients. *Diabet Med* 26:437–441 . doi: 10.1111/j.1464-5491.2009.02690.x
37. Pearson ER, Pruhova S, Tack CJ, et al (2005) Molecular genetics and phenotypic characteristics of MODY caused by hepatocyte nuclear factor 4alpha mutations in a large European collection. *Diabetologia* 48:878–885 . doi: 10.1007/s00125-005-1738-y
38. Pearson ER, Starkey BJ, Powell RJ, et al (2003) Genetic cause of hyperglycaemia and response to treatment in diabetes. *Lancet* 362:1275–1281 . doi: 10.1016/S0140-6736(03)14571-0
39. Smith RJ, Nathan DM, Arslanian SA, et al (2010) Individualizing Therapies in Type 2 Diabetes Mellitus Based on Patient Characteristics: What We Know and What We Need to Know. *J Clin Endocrinol Metab* 95:1566–1574 . doi: 10.1210/jc.2009-1966
40. Picard S, Titier K, Etienne G, et al (2007) Trough imatinib plasma levels are associated with both cytogenetic and molecular responses to standard-dose imatinib in chronic myeloid leukemia. *Blood* 109:3496–3499 . doi: 10.1182/blood-2006-07-036012
41. Chen D-Y, Chen Y-M, Hsieh T-Y, et al (2016) Drug trough levels predict therapeutic responses to dose reduction of adalimumab for rheumatoid arthritis patients during 24 weeks of follow-up. *Rheumatology (Oxford)* 55:143–148 . doi: 10.1093/rheumatology/kev298
42. Kupfer DJ, Hanin I, Spiker DG, et al (1977) Amitriptyline plasma levels and clinical response in primary depression. *Clinical Pharmacology & Therapeutics* 22:904–911 . doi: 10.1002/cpt1977226904
43. Bartelds GM, Wijbrandts CA, Nurmohamed MT, et al (2007) Clinical response to adalimumab: relationship to anti-adalimumab antibodies and serum adalimumab concentrations in rheumatoid arthritis. *Ann Rheum Dis* 66:921–926 . doi: 10.1136/ard.2006.065615

44. Bartelds GM, Krieckaert CLM, Nurmohamed MT, et al (2011) Development of antidrug antibodies against adalimumab and association with disease activity and treatment failure during long-term follow-up. *JAMA* 305:1460–1468 . doi: 10.1001/jama.2011.406
45. Radstake TRDJ, Svenson M, Eijsbouts AM, et al (2009) Formation of antibodies against infliximab and adalimumab strongly correlates with functional drug levels and clinical responses in rheumatoid arthritis. *Ann Rheum Dis* 68:1739–1745 . doi: 10.1136/ard.2008.092833
46. Rosenstock J, Zinman B (2007) Dipeptidyl peptidase-4 inhibitors and the management of type 2 diabetes mellitus. *Curr Opin Endocrinol Diabetes Obes* 14:98–107 . doi: 10.1097/MED.0b013e3280a02f65
47. Inzucchi SE, McGuire DK (2008) New drugs for the treatment of diabetes: part II: Incretin-based therapy and beyond. *Circulation* 117:574–584 . doi: 10.1161/CIRCULATIONAHA.107.735795
48. Ahrén B (2007) Dipeptidyl peptidase-4 inhibitors: clinical data and clinical implications. *Diabetes Care* 30:1344–1350 . doi: 10.2337/dc07-0233
49. Drucker DJ, Nauck MA (2006) The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet* 368:1696–1705 . doi: 10.1016/S0140-6736(06)69705-5
50. Bergman A, Mistry GC, Luo W-L, et al (2007) Dose-proportionality of a final market image sitagliptin formulation, an oral dipeptidyl peptidase-4 inhibitor, in healthy volunteers. *Biopharm Drug Dispos* 28:307–313 . doi: 10.1002/bdd.559
51. European Medicines Agency. (2016) Januvia 25 mg film-coated tablets: summary of product characteristics. In: <http://www.ema.europa.eu/>
52. Bergman A, Ebel D, Liu F, et al (2007) Absolute bioavailability of sitagliptin, an oral dipeptidyl peptidase-4 inhibitor, in healthy volunteers. *Biopharm Drug Dispos* 28:315–322 . doi: 10.1002/bdd.560
53. Vincent SH, Reed JR, Bergman AJ, et al (2007) Metabolism and excretion of the dipeptidyl peptidase 4 inhibitor [14C]sitagliptin in humans. *Drug Metab Dispos* 35:533–538 . doi: 10.1124/dmd.106.013136
54. Migoya EM, Stevens CH, Bergman AJ, et al (2009) Effect of moderate hepatic insufficiency on the pharmacokinetics of sitagliptin. *Can J Clin Pharmacol* 16:e165-170

55. Merck Sharp & Dohme Corp Januvia_ (sitagliptin) tablets: US prescribing information. In: https://www.merck.com/.https://www.merck.com/product/usa/pi_circulars/j/januvia/januvia_mg.pdf
56. Bergman AJ, Cote J, Yi B, et al (2007) Effect of renal insufficiency on the pharmacokinetics of sitagliptin, a dipeptidyl peptidase-4 inhibitor. *Diabetes Care* 30:1862–1864 . doi: 10.2337/dc06-2545
57. Bain S, Eligar V (2013) A review of sitagliptin with special emphasis on its use in moderate to severe renal impairment. *Drug Design, Development and Therapy* 893 . doi: 10.2147/DDDT.S32331
58. Andersen ES, Lund A, Bagger JI, et al (2018) Is glucagon-like peptide-1 fully protected by the dipeptidyl peptidase 4 inhibitor sitagliptin when administered to patients with type 2 diabetes? *Diabetes Obes Metab*. doi: 10.1111/dom.13321
59. Alba M, Sheng D, Guan Y, et al (2009) Sitagliptin 100 mg daily effect on DPP-4 inhibition and compound-specific glycemic improvement. *Curr Med Res Opin* 25:2507–2514 . doi: 10.1185/03007990903209514
60. Kim D, Wang L, Beconi M, et al (2005) (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine: a potent, orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. *J Med Chem* 48:141–151 . doi: 10.1021/jm0493156
61. Iwamoto Y, Taniguchi T, Nonaka K, et al (2010) Dose-ranging efficacy of sitagliptin, a dipeptidyl peptidase-4 inhibitor, in Japanese patients with type 2 diabetes mellitus. *Endocr J* 57:383–394
62. Proks P, Reimann F, Green N, et al (2002) Sulfonylurea stimulation of insulin secretion. *Diabetes* 51 Suppl 3:S368-376
63. Kobayashi K, Kimura M, Sakoguchi T, et al (1981) Influence of blood proteins on biomedical analysis. III. Pharmacokinetics and protein binding of gliclazide. *J Pharmacobio-dyn* 4:436–442
64. Campbell DB, Adrianessens P, Hopkins YW, Gordon B, Williams JRB (1980) Pharmacokinetics and metabolism of gliclazide: A review. *Academic Press and the Royal Society of Medicine* 71–82
65. Shiba T, Kajinuma H, Suzuki K, et al (1986) Serum gliclazide concentration in diabetic patients. Relationship between gliclazide dose and serum concentration. *Diabetes Res Clin Pract* 2:301–306

66. Oida T, Yoshida K, Kagemoto A, et al (1985) The metabolism of gliclazide in man. *Xenobiotica* 15:87–96
67. Biswal S, Sahoo J, Murthy PN (2009) Physicochemical Properties of Solid Dispersions of Gliclazide in Polyvinylpyrrolidone K90. *AAPS PharmSciTech* 10:329–334 . doi: 10.1208/s12249-009-9212-7
68. Forette B, Rolland A, Hopkins Y, Gordon B, Campbell B. (1982) Gliclazide pharmacokinetics in the elderly. *Excerpta Medica, Nairobi*, pp 8–9
69. Inzucchi SE, Bergenstal RM, Buse JB, et al (2012) Management of Hyperglycemia in Type 2 Diabetes: A Patient-Centered Approach: Position Statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care* 35:1364–1379 . doi: 10.2337/dc12-0413
70. Garber AJ, Abrahamson MJ, Barzilay JI, et al (2015) AACE/ACE comprehensive diabetes management algorithm 2015. *Endocr Pract* 21:438–447 . doi: 10.4158/EP15693.CS
71. Groop LC (1992) Sulfonylureas in NIDDM. *Diabetes Care* 15:737–754
72. Groop LC, Ratheiser K, Luzi L, et al (1991) Effect of sulphonylurea on glucose-stimulated insulin secretion in healthy and non-insulin dependent diabetic subjects: a dose-response study. *Acta Diabetol* 28:162–168
73. Goldberg RB, Holvey SM, Schneider J (1996) A dose-response study of glimepiride in patients with NIDDM who have previously received sulfonylurea agents. The Glimepiride Protocol #201 Study Group. *Diabetes Care* 19:849–856
74. Simonson DC, Kourides IA, Feinglos M, et al (1997) Efficacy, safety, and dose-response characteristics of glipizide gastrointestinal therapeutic system on glycemic control and insulin secretion in NIDDM. Results of two multicenter, randomized, placebo-controlled clinical trials. The Glipizide Gastrointestinal Therapeutic System Study Group. *Diabetes Care* 20:597–606
75. Stenman S, Melander A, Groop PH, Groop LC (1993) What is the benefit of increasing the sulfonylurea dose? *Ann Intern Med* 118:169–172

Chapter 2: Data Source and Methods

Data Source: *The MASTERMIND Randomised Control Crossover Trial (NCT01847144)*

The MASTERMIND pilot study is an open label, randomized, two arm crossover trial conducted in the South West of England and Dundee, Scotland (ClinicalTrials.gov clinical trials identifier NCT01847144). This pilot trial was conducted to inform and design further studies into the inter-individual variation in therapeutic response to glucose lowering therapy.

2.1 Study Procedures and Flow

2.1.1 Screening and Identification

Potential recruits, who previously consented to be contacted about research projects, were identified from existing research databases. A covering letter that outlined the project was sent to these individuals by a Data Manager. Where potential recruits were identified via primary or secondary care, their clinician provided a brief written outline of the project and instructions and the contact information of the research team.

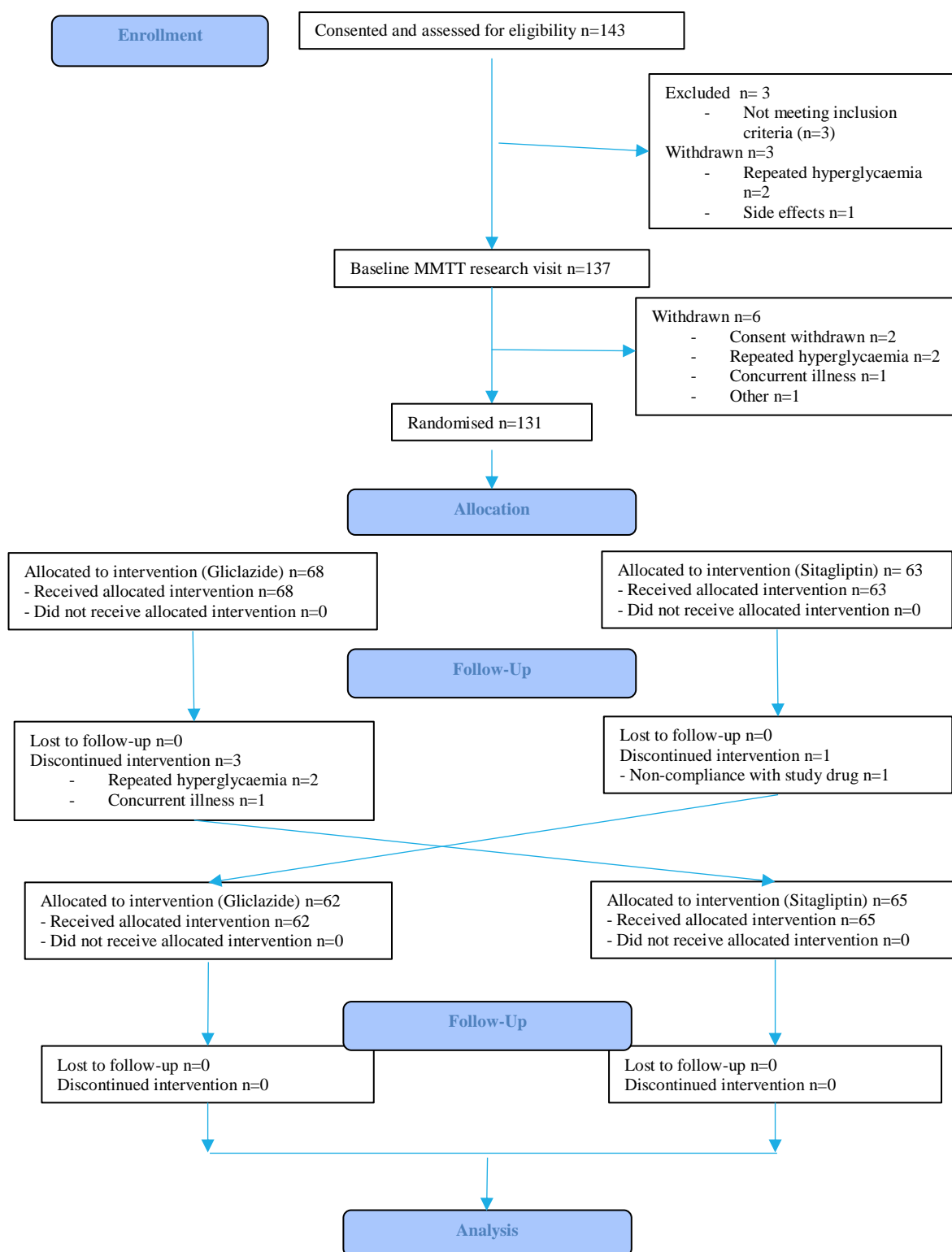
Recruitment

Inclusion/exclusion criteria is displayed in *Table 1*. Prior to recruitment, all potential participants were provided with written information and an opportunity to discuss the project with a member of the research team. All participants recruited to the study were required to give written informed consent and were excluded from the recruitment scheme if they lacked capacity.

Inclusion Criteria	Exclusion criteria
<ul style="list-style-type: none"> Age >18 and <80 	<ul style="list-style-type: none"> Current treatment includes: insulin, GLP-1 agonists, DPP-IV inhibitors, glinides, SGLT2 inhibitors
<ul style="list-style-type: none"> Clinical diagnosis of Type 2 Diabetes 	<ul style="list-style-type: none"> Renal impairment (eGFR <30 ml/min/1.73m²)
<ul style="list-style-type: none"> Currently treated with sulphonylurea tablets 	<ul style="list-style-type: none"> Active infection (any infection requiring antibiotics at present)
<ul style="list-style-type: none"> No change in diabetes treatment (new treatments or dose change) within last 3 months 	<ul style="list-style-type: none"> Active foot ulcer
<ul style="list-style-type: none"> Last HbA1c (taken within last 12 months) of ≥42 mmol/mol and ≤75 mmol/mol (6-9%) 	<ul style="list-style-type: none"> Recent (within 3 months) surgery or planned surgery
<ul style="list-style-type: none"> Able and willing to monitor home blood glucose 	<ul style="list-style-type: none"> Cardiovascular disease (angina, myocardial infarction, stroke, transient ischemic episode) occurring within the previous 3 months
<ul style="list-style-type: none"> Able and willing to give informed consent 	<ul style="list-style-type: none"> Previous history of pancreatitis
	<ul style="list-style-type: none"> Pregnant, breastfeeding or planning a pregnancy over the study period
	<ul style="list-style-type: none"> Unable/unwilling to monitor home blood glucose

Table 1- Inclusion/Exclusion criteria

Figure 1- MASTERMIND consort diagram



Study Visits

Of 143 patients that were initially eligible and gave consent, 137 participants were recruited and took part in the initial period of sulphonylurea withdrawal (part 1); All participants were invited to take part in the follow on (part 2), of which 131 completed this stage. The second period of the study examined glycaemic response to 2 different treatments. A flow diagram of study visits is shown in *Figure 2*.

2.2 Part One: Visit 1: Baseline

Following informed written consent, a fasting blood sample (approximately 35 mls) was obtained for the biochemical analysis, including: saved serum and plasma, HbA1c, glycosylated albumin, glucose, C-peptide, liver function tests (ALT, Bilirubin, GGT, albumin), renal function, fasting lipid profile, pancreatic autoantibodies (GAD and IA2) and medication levels.

Baseline data for the following biological/clinical parameters were also obtained, including: age, age at diagnosis, duration of diabetes, height, weight, waist-hip ratio, % body fat, ethnicity, current treatment and co-morbidities.

Treatment Cessation and Monitoring

Following baseline assessment, participants discontinued their sulphonylurea treatment.

Visit 2: Mixed Meal Tolerance Test (location: research centre)

At 1 week participants attended the research facility fasted, having withheld all other morning diabetes medication. Prior to receiving the meal test a urine sample was collected and then collected again after 2 hours. Fasted blood samples were obtained for the following measurements: plasma glucose, fructosamine (a shorter term measure of glycaemia than HbA1c), C-peptide, insulin, and saved serum and plasma. Following this, a liquid meal of Fortisip 250mls (mixed meal tolerance test (MMTT)) was administered and blood samples were taken every 30 minutes for 2 hours. Blood samples were analysed for saved serum and plasma, and for measurement of C-peptide, insulin and glucose. At the end of the test, breakfast was provided and participants took their usual morning diabetes medication.

Visit 3: Assessment of Glycaemia

At 2 weeks, a fasting blood sample was obtained for the same biochemical analysis (glycosylated albumin, glucose, saved plasma & serum, HbA1c) and the

participants' weight was repeated. Following the 2 week blood sample, participants had the option to continue to part 2 of the study, or withdraw from the study. Of the 137 who completed part 1, 131 participants continued to part 2. Reasons for discontinuation can be found in the CONSORT diagram (*Figure 1*). For participants with hyperglycaemia that required recommencing their treatment, they had option of participating part 2 in place of restarting usual treatment.

2.3 Part 2: Randomisation and Adherence

At visit 3 participants undertaking part 2 of the study were randomised to 4 weeks of treatment of Sitagliptin 100mg or Gliclazide 80mg once daily, taken in the morning. Standardized doses of the medications were given in MEMS caps containers. MEMS caps containers record opening times, and are the gold standard medication adherence. To measure physical activity participants wore an accelerometer for 1 week prior to the 4 week mixed meal assessment in each treatment arm.

Visit 4: Mixed Meal Tolerance Test (location: research centre)

Following 4 weeks of the first treatment, participants attended the research facility fasting and had their physical parameters (weight, height, waist circumference) measured as in previous visits, followed by a MMTT. The mixed meal test was performed in the same way as visit 2, except with additional samples obtained at 3 and 4 hours. The additional samples at 3 and 4 hours allowed for drug concentrations to be measured for pharmacokinetic studies. Study medication was administered prior to the test but any other medication was taken after the mixed meal test with a provided lunch. Following this, participants were asked to stop taking their randomised study treatment for a two week washout period (but were allowed to take other medication with the exception of sulphonylurea).

Visit 5: Assessment of glycaemia and initiation of second study treatment

After the two week washout period, a further fasting blood sample was obtained for analysis of: HbA1c, glycosylated albumin, fasting glucose and stored serum and plasma. Afterwards, participants were then given 4 weeks of the alternative treatment (participants who initially took sitagliptin will take gliclazide and vice-versa).

Visit 6: Mixed Meal Tolerance Test (location: research centre)

After the 4 week treatment period, participants attended the research facility fasted for a further mixed meal test and, weight and HbA1c measurement as per visit 4. Participants then discontinued the study medication (washout period) for two weeks.

Visit 7: Assessment of Glycaemia

After the two week washout period, where participants took their usual medication without sulphonylurea/study medication, a further fasting blood test was obtained for the same biochemical data listed above. The study concluded at this point and participants' usual sulphonylurea therapy was restarted.

Randomisation

The randomised order of treatments received by participants for cross-over section of the study (Part 2), was calculated using a randomisation table (Stats Direct) and was overseen by the clinical research facility statistician.

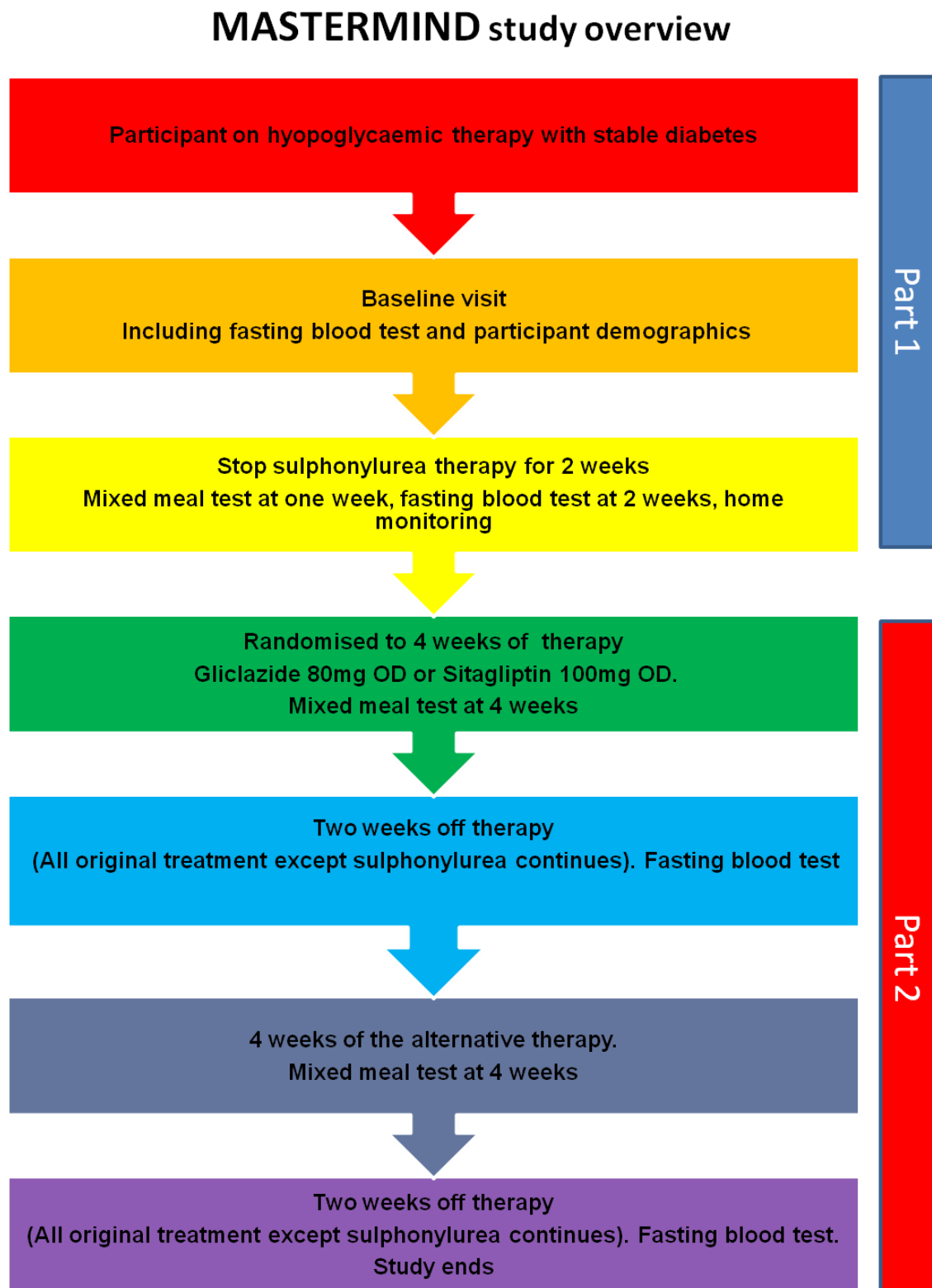
Concomitant Therapies

Participants were allowed to continue all non-diabetes related medication and were asked not to change diabetes treatment (outside the study protocol changes) for the duration of the study.

Pregnancy

A pregnancy test was offered to pre-menopausal women to ensure as pregnancy is an exclusion criteria for this study.

Figure 2 - MASTERMIND Study overview- obtained directly from MASTERMIND Study Protocol.



Subject Withdrawal

Subjects were informed of their right to withdraw from the study at any time up until the samples and data were coded but not anonymised. If participants withdrew after samples were anonymised, their samples and any data was retained and used in the analysis. For participants who permanently withdrew from the study, or were lost to follow-up, the reason was recorded (*Figure 1: MASTERMIND CONSORT flow diagram*).

Ethics and Regulatory Approvals

The study was conducted in compliance with the principles of the Declaration of Helsinki (1996), the principles of GCP and in accordance with all applicable regulatory requirements. The study was approved by the South West Research Ethics Committee (REC).

2.4 Understanding the study design

Crossover Design

The optimum method for investigating the variation in therapeutic response to Type 2 Diabetes remains unclear. However, crossover study designs, where each participant receives each treatment for a single treatment period, have the capacity to identify whether differences between treatments or between individuals contribute to the wide variation in therapeutic response to medications.

Advantages of the crossover design

One advantage of a crossover design is that participants are involved in both intervention arms hence serve as their own control. This not only reduces inter-individual variability (as comparisons of different treatments are performed on the same individual) but also reduces the sample size needed to obtain sufficient power. The smaller sample size means study costs are often lower when compared with the more traditional parallel group study design. The stable and chronic nature of T2D, its measurable glycaemic endpoints and multiple treatment intensification options make T2D an ideal candidate for crossover trials for stratified research in this area.

Washout period

Another advantage of this crossover design is that it allows the study of glycaemic response associated with treatment withdrawal, this can be obtained from a washout period, where all study medication is withheld. If change in glycaemia after stopping medication is able to predict response on treatment, then this finding would have positive implications for clinical practice. Data from a sulphonylurea withdrawal trial conducted over a 4 month period suggested, that glycaemic response measurement following 3-4 weeks of withdrawal as a robust measurement of therapeutic response[1]. Treatment withdrawal data is useful as it can be compared to data from the initial glycaemic response to treatment and is a cost effective method to establish if therapeutic response is sustained and reproducible. Furthermore, it reduces changes in lifestyle or adherence that are associated with commencing a new medication in a clinical or trial setting. Despite this, before changes in glycaemic response secondary to treatment withdrawal is assessed, non-response due to lack of adherence needs to be investigated.

Assessment of glycaemic response.

HbA1c (glycosylated haemoglobin) is the gold standard measurement of glycaemic control and reflects glycaemia over a 3 month period. Despite this, studies investigating inter-individual variation in treatment response may not require a measurement of the full glycaemic response (via HbA1c). Instead a measurement of that is proportional to an individual's maximum therapeutic response may be sufficient, such as fructosamine. Fructosamine is a measure glycosylated albumin which glycaemia over a shorter time period and has high inter-individual validity (however is less valid compared to HbA1c) [2]. Furthermore, there is trial data to suggest the majority of glycaemic change associated with most diabetes agents, occurs within the first few weeks of commencing therapy [3–7].

2.5 References

1. Landstedt-Hallin L, Arner P, Lins PE, et al (1999) The role of sulphonylurea in combination therapy assessed in a trial of sulphonylurea withdrawal. Scandinavian Insulin-Sulphonylurea Study Group Research Team. *Diabet Med* 16:827–834
2. Takahashi S, Uchino H, Shimizu T, et al (2007) Comparison of glycated albumin (GA) and glycated hemoglobin (HbA1c) in type 2 diabetic patients: usefulness of GA for evaluation of short-term changes in glycemic control. *Endocr J* 54:139–144
3. Goldstein BJ, Feinglos MN, Luncford JK, et al (2007) Effect of initial combination therapy with sitagliptin, a dipeptidyl peptidase-4 inhibitor, and metformin on glycemic control in patients with type 2 diabetes. *Diabetes Care* 30:1979–1987 . doi: 10.2337/dc07-0627
4. Iwamoto Y, Taniguchi T, Nonaka K, et al (2010) Dose-ranging efficacy of sitagliptin, a dipeptidyl peptidase-4 inhibitor, in Japanese patients with type 2 diabetes mellitus. *Endocr J* 57:383–394
5. Nonaka K, Kakikawa T, Sato A, et al (2008) Efficacy and safety of sitagliptin monotherapy in Japanese patients with type 2 diabetes. *Diabetes Res Clin Pract* 79:291–298 . doi: 10.1016/j.diabres.2007.08.021
6. DeFronzo RA, Goodman AM (1995) Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. The Multicenter Metformin Study Group. *N Engl J Med* 333:541–549 . doi: 10.1056/NEJM199508313330902
7. Ferrannini E, Ramos SJ, Salsali A, et al (2010) Dapagliflozin monotherapy in type 2 diabetic patients with inadequate glycemic control by diet and exercise: a randomized, double-blind, placebo-controlled, phase 3 trial. *Diabetes Care* 33:2217–2224 . doi: 10.2337/dc10-0612

Chapter 3

Chapter 3: Precision medicine in Type 2 Diabetes: is variation in response to sitagliptin and gliclazide therapy related to drug levels?

N.B This chapter is being submitted as an original research paper to the journal 'Diabetes, Obesity and Metabolism' for publication.

3.1 Introduction

Trial and observational data show a wide variation in glycaemic response to glucose lowering therapy [1–7]. While much of this variation may be related to adherence and, change in lifestyle, there is increasing evidence that individual's biological characteristics are associated with variation in therapeutic response[8] Understanding the mechanisms underpinning response variation and identifying robust predictors of response may allow a precision medicine (or stratified) approach to type 2 diabetes treatment, where therapy choice informed by an individual's likely response or side effect risk, thus minimising adverse effects and maximising therapeutic benefit.[9] Additionally understanding reasons for variation in drug response could aid in dose selection and inform the development of new medications with lower non-response rates.

A key candidate that may influence response to treatment is variation in achieved drug concentrations. In diabetes it is standard practice to use a single dose of a drug, regardless of patient characteristics; for example patients weighing 40kg and 140kg would have an identical dose for most glucose lowering medications. It is therefore likely that different individuals have different exposure to a drug; a potential cause of variation in response to therapy and side effect risk. Plasma drug concentrations have been shown to be associated with therapeutic response to treatment in several other diseases[10–12]; however whether variations in plasma drug levels are associated with variation in glycaemic response in T2D is not known.

We aimed to investigate whether variation in plasma drug concentrations of sitagliptin 100mg OD and gliclazide 80mg OD (two of the most commonly prescribed agents after metformin in T2D)[13] are associated with biological characteristics of individuals, and whether variation in plasma drug concentrations is a major cause of variation in glucose lowering response to therapy.

3.2.1 Study Design

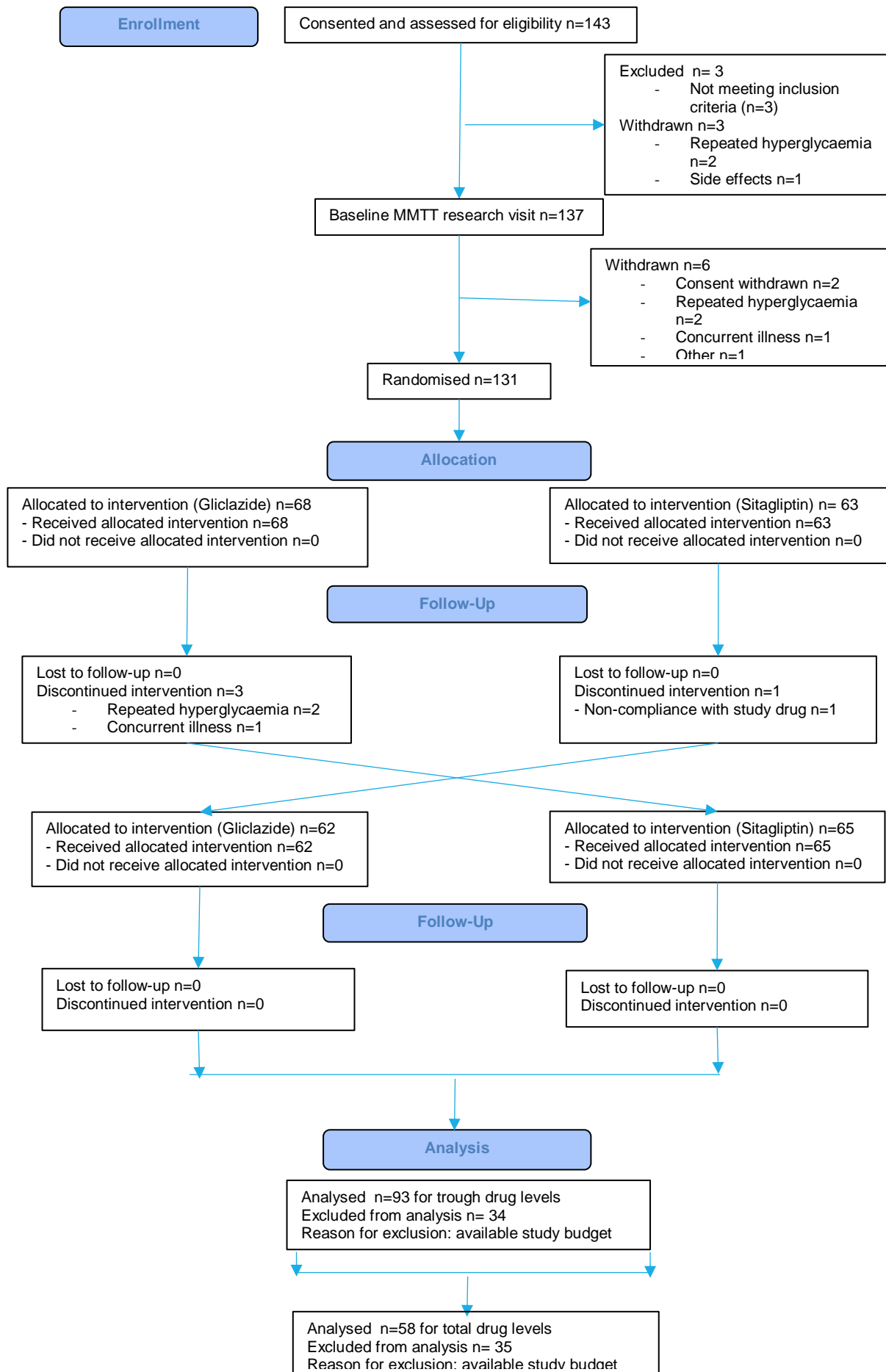
We assessed the relationship between blood plasma gliclazide and sitagliptin levels, participant characteristics and glycaemic response in the MASTERMIND pilot study, an open label, randomized, two arm crossover trial conducted in the South West of England and Dundee, Scotland (ClinicalTrials.gov clinical trials identifier NCT01847144). This pilot trial was conducted to inform and design further studies into the inter-individual variation in therapeutic response to glucose lowering therapy.

3.2.2 Recruitment

Potential recruits were contacted from existing clinical research databases, where participants had provided consent to being contacted about future research projects. Of 143 participants who consented to the study 68 were randomized to treatment sequence AB (gliclazide = A and sitagliptin =B) and 63 were randomized to treatment sequence BA. 127 participants completed both arms of the trial (*Figure 3* CONSORT flow diagram).

Inclusion criteria were: a clinical diagnosis of Type 2 diabetes and were treated with sulphonylurea (SU) therapy (with or without metformin), age at recruitment >18 and <80 years, no change in diabetes treatment (new treatments or dose change) within last 3 months, HbA1c of ≥ 42 mmol/mol and ≤ 75 mmol/mol (≥ 6 - $\leq 9\%$). Exclusion criteria were: renal impairment (eGFR <30 ml/min/1.73m²), active infection requiring antibiotics, active foot ulcer, cardiovascular disease occurring within the previous 3 months and pregnancy/breastfeeding.

Figure 3- Mastermind CONSORT flow diagram



3.2.2 Study procedure

At enrolment, participants discontinued prior sulphonylurea (SU) therapy for 2 weeks. 7 days after withdrawal of SU therapy, participants attended fasting and withheld morning medication for a mixed meal tolerance test (MMTT). Following baseline sample collection a liquid mixed meal (250ml of Fortisip Compact (Nutricia, Trowbridge, UK)) was consumed and blood samples were collected every 30 minutes for 2 hours for glucose measurement.

At the end of the two-week washout period, participants were randomised into two groups, where they either received a standard dose of gliclazide (80mg) or sitagliptin (100mg) each in a random order. Study medication was taken once daily in the morning for a period of 4 weeks; between each treatment period participants observed a washout period of 2 weeks (*Figure 4*).

At the end of each treatment period participants attended the clinical research facility in the morning fasting, having withheld their morning glucose lowering medication. Following baseline blood sampling, participants took their study medication immediately followed by a mixed meal tolerance test. Blood samples were taken every 30 minutes for 2 hours, with additional sample collections at 3 and 4 hours.

All blood samples were stored at -80°C prior to analysis. Where participants were treated with metformin, this was withheld at all mixed meal test visits and administered on completion of sample collection.

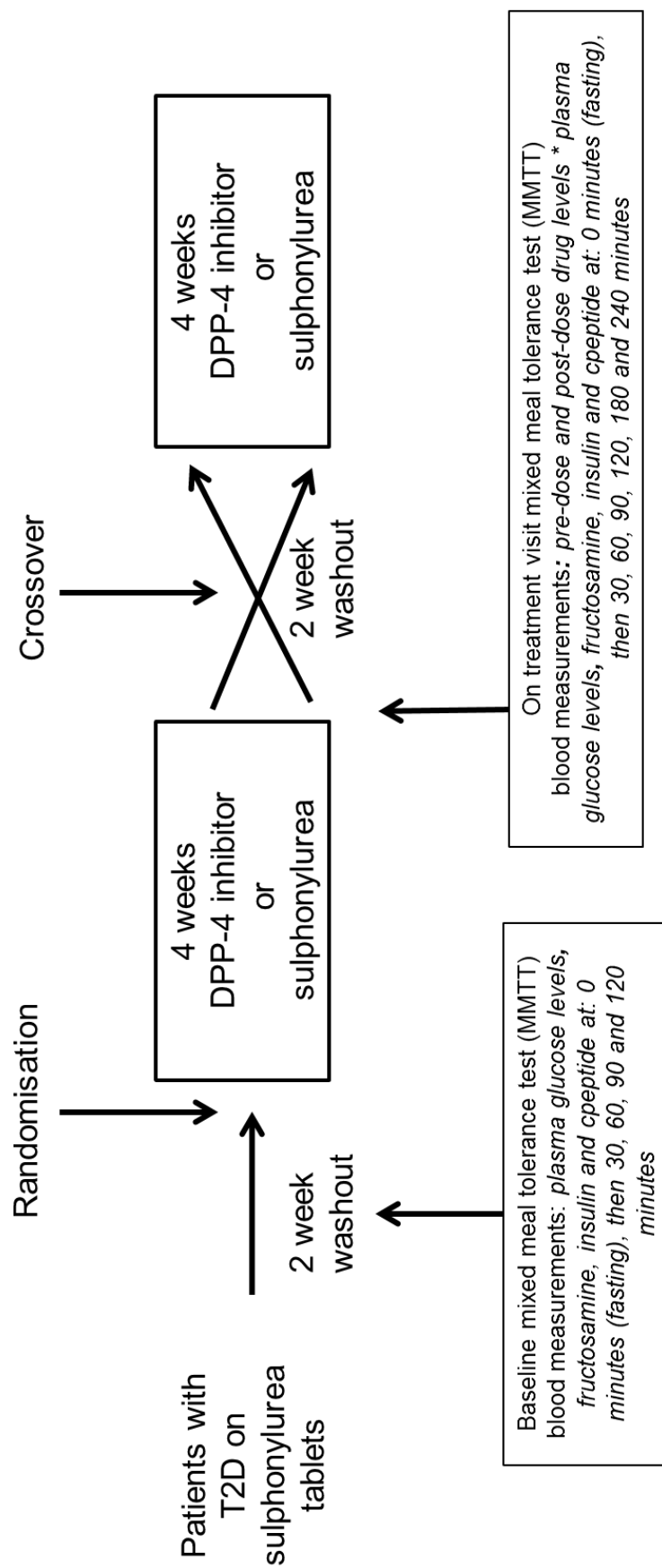


Figure 4 - Study design. Participants withdrew sulphonylurea therapy for up to 2 weeks followed by an optional cross-over extension where they were randomized to 4 weeks of treatment with gliclazide (sulphonylurea) or sitagliptin (DPP-4 inhibitor), followed by 4 weeks of the second treatment with a 2 week wash-out period in between.
 *Study medication was administered 15 minutes after commencing MMTT.

3.2.4 Randomisation

Randomisation for the cross-over section of the study and for the order of treatments received by participants was calculated using a randomisation table (Stats Direct, Cambridge, United Kingdom) overseen by the clinical research facility statistician.

3.2.5 Biochemical analysis: Measurement of drug levels

Analysis for plasma levels of sitagliptin and gliclazide was performed by Covance Laboratories (Covance Laboratories Limited, Harrogate, UK) by Protein Precipitation followed by Liquid Chromatography with Tandem Mass Spectrometric Detection (LC MS/MS).

Other laboratory measurements

Other laboratory analysis was undertaken by the Academic Department of Blood Sciences at the Royal Devon and Exeter NHS Foundation Trust, Exeter, UK. Glucose was measured by enzymatic colorimetric assay GOD-PAP using a Roche (Manheim, Germany) MODULAR P800 analyser. Creatinine was measured photometrically by Jaffé method using a Roche MODULAR P analyser. Fructosamine was measured using the Randox (Belfast, UK) enzymatic kit on the Roche Modular P800 analyser.

3.2.6 Statistical analysis

Statistical analysis was performed using STATA (StataCorp. 2015. Stata Statistical Software: Release 14.2 College Station, TX: StataCorp LP.). Data were assessed visually for distribution and where results are non-normally distributed, results are expressed as median(IQR). All model assumptions were confirmed prior to all analysis.

Assessing pharmacokinetic and glycaemic parameters

Individual pharmacokinetic and pharmacodynamics parameters for sitagliptin and gliclazide calculated include: time to maximum concentration (T_{max}), maximum drug concentration achieved (C_{max}), peak plasma concentration and total area under curve at 240 minutes ($AUC_{0-240min}$) levels.

Fasting, incremental $AUC_{0-240min}$ (post meal glucose) and fructosamine glycaemic response were calculated for both sitagliptin and gliclazide and change from baseline was also calculated. For normally distributed results, comparison of change in glycaemic response between each drug was calculated using a paired

T-test. All AUC pharmacokinetic and glycaemic parameters were calculated using the trapezoid rule.

Calculation of period/carryover effect

The period effect (calculated by comparing the mean difference between sitagliptin and gliclazide treatment in the group starting on sitagliptin with that in the group starting on gliclazide) and the carryover effect (comparing baseline values for each treatment group) were calculated using Student's t test.

Assessing relationship between plasma drug concentrations and therapeutic response

We assessed the relationship between drug levels (trough and total AUC_{0-240min}) and glycaemic outcomes (on therapy fasting glucose, incremental AUC_{0-240min} and fructosamine) using linear regression. All linear regression models were adjusted for the baseline glucose measurement at initial MMTT visit (fasting, incremental AUC₀₋₁₂₀ or fructosamine respectively). Analysis of incremental AUC_{0-240min} was adjusted for the 2-hour incremental AUC glucose at baseline. Additionally, as a significant period effect was observed across glucose measurements (see *Supplementary Table 1 and 2*) all linear regression models were adjusted for drug order.

While HbA1c was measured, a marked carryover effect was noted (effect(95%CI) -3.1 (-3.95, -2.24), $p < 0.0001$) and therefore no further assessment of HbA1c was performed.

To assess whether participants with very low drug levels has reduced glycaemic response, we performed a secondary categorical analysis, comparing glycaemic response in participants with trough drug levels <25th centile with the remaining participants. This analysis was performed using ANCOVA with the baseline measurement of the glycaemic endpoint and drug order as covariates.

Assessing relationship between plasma concentrations and biological characteristics

The association of BMI, weight, eGFR, age and gender and circulating drug levels (trough, total AUC_{0-240min} and peak) were assessed using a univariable linear regression model; with plasma drug concentrations as fixed outcomes and the respective biological parameters as covariates. Whether age and eGFR were independently associated with drug levels was assessed using multivariable linear regression.

3.3 Results

Participant characteristics

Trough plasma drug levels were measured in 93 of 127 study participants who completed the study, per protocol with sample collection at all time points at the time analysis was conducted. Post MMTT drug levels were measured at all time points in 58 of these participants. The number of samples measured was determined on the basis of order of recruitment and by the available study budget. Baseline characteristics are displayed in *Table 2* for participants with trough drug levels measured (n=93) and for participants with post dose area under the curve drug level (n=58). 91% of those with trough drug levels were metformin treated, had a mean diabetes duration of 10 years, mean BMI 30.8 kg/m² and 70% were male. Mean baseline glucose in these participants was 9.3 mmol/L fasting and mean baseline incremental AUC 417.4 mmol/L.

Baseline Characteristics	Participants who completed the study per protocol (n=127)	Participants with trough drug levels measured (n=93)	Participants with total AUC drug levels measured (n=58)
Age (years)	69 (65-74)	69 (65-74)	70 (65-74)
Ethnicity % white (n)	96.1(122)	97(90)	95(55)
Gender, % male (n)	70.8 (89)	70.01 (89)	74.1 (43)
Diabetes duration (years)	10 (7-13)	10 (6-13)	11 (6-14)
Weight (kg)	87.8 (80-97.9)	88.8 (79.4-98.9)	90.2 (80-99.8)
BMI (kg/m ²)	31.2 (28.4 -34.5)	30.8 (28.2-34.4)	31.3 (28 -34)
eGFR (mL/min/1.73 m ²)	70 (60-85)	75 (63-86)	76 (63-85)
Baseline Fasting Glucose (mmol/L)	9.4 (8.3-11.5)	9.3 (8.2-10.7)	9.5 (8.5-11.5)
Baseline Incremental AUC 120 * Glucose (mmol/L)	414(326-501)	414(326-501)	430(326-501)
Baseline Fructosamine (umol/L)	350 (310-394)	344 (300 -378)	347 (298-381)
Proportion of participants treated with Metformin (%)	91	91	91

Table 2- baseline characteristics of participants. Results expressed as median (IQR) unless specified otherwise.

Pharmacokinetics

Time to maximum plasma concentration (T_{max}) was 180 minutes for sitagliptin (Figure 5a). Maximum plasma concentration (C_{max}) of sitagliptin was median (IQR) 393.5 (112– 482) ng/mL. T_{max} was not captured with gliclazide as plasma concentrations were still increasing after 4 hours post MMTT (Figure 5b).

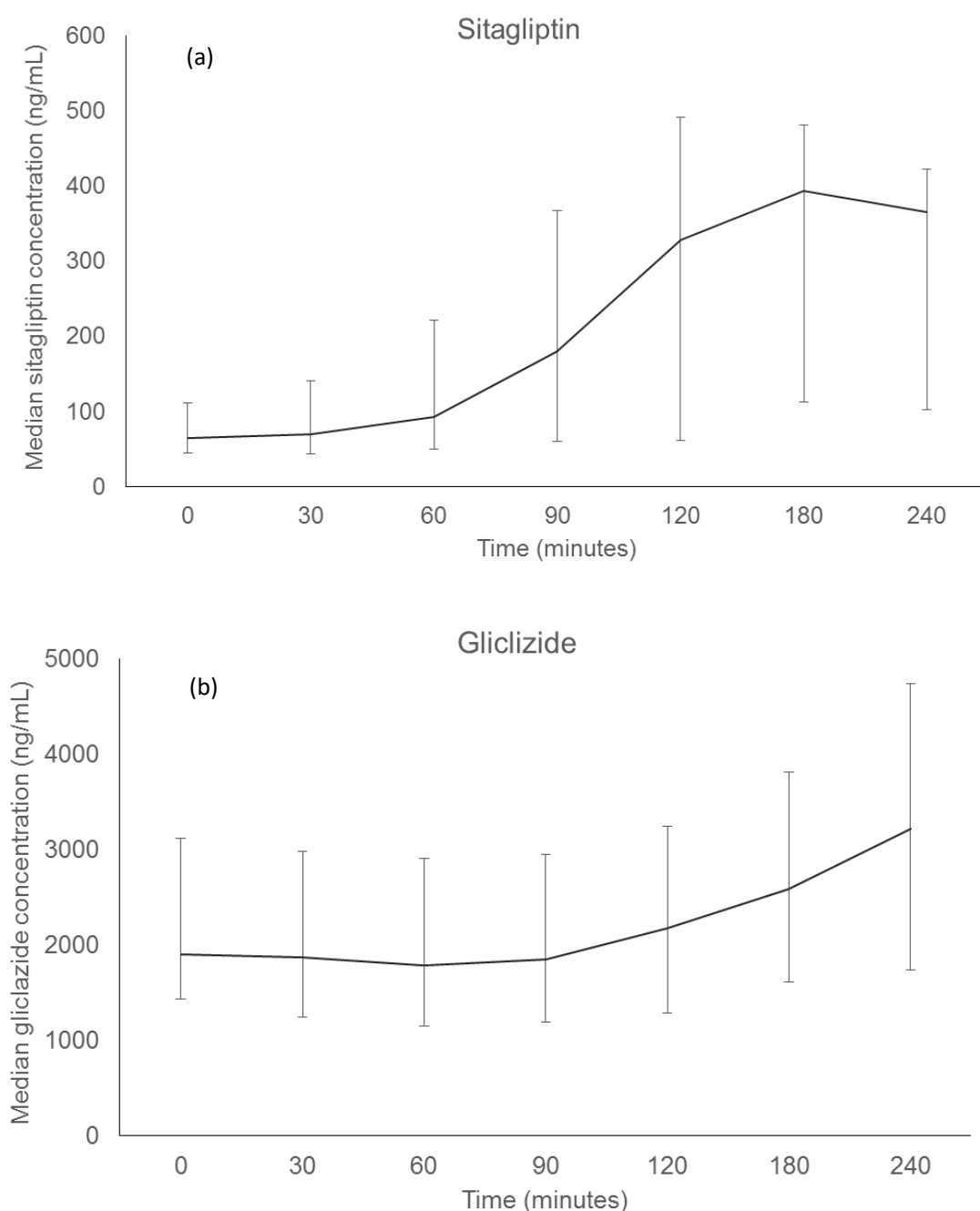


Figure 5- Line graph (with error bars representing the interquartile range) showing median plasma drug level of sitagliptin (a) and gliclazide (b) over time in on treatment mixed meal tolerance tests. Daily dose given immediately after time 0.

Glycaemic response to therapy

Mean (SD) change in fasting glucose was lower with sitagliptin compared to gliclazide; -0.04 (1.59) vs -1.3 (1.74) mmol/L, $p < 0.01$ respectively. In contrast, the average change in 2 hour incremental AUC_{0-240min} glucose was greater with sitagliptin than gliclazide: mean (SD) -47.19 (138.4) mmol/L vs -6.4 (115.2) mmol/L, $p < 0.0001$. Mean (SD) fructosamine change from baseline was +7.2 (40.5) μ mol/L for sitagliptin and -18.8 (43.3) μ mol/L for gliclazide, $p < 0.01$.

Period and carryover effect

There was no evidence of carryover effect influencing glucose response, however there was a significant period effect across most glucose measurements, fasting glucose effect(95%CI): -0.35 (-0.77, 0.06), $p = 0.1$, HbA1c effect(95%CI): -3.1 (-3.95, -2.24) $p < 0.001$ and fructosamine effect(95%CI): -18.5 (-27.62, -9.38), $p < 0.001$ (*Supplementary Table 1 and 2 – see page 79*).

Drug plasma concentrations were highly variable despite a standard dose regimen

Trough plasma concentrations were highly variable with a 142.5 fold variation in trough levels of sitagliptin range(median) 3.7-523 (8.9) ng/mL and a 9.4 fold variation in trough levels of gliclazide range(median) 832-7780 (1875) ng/mL. High variation was also seen with total AUC_{0-240min} plasma drug concentrations. There was a 39.4 fold variation in total AUC_{0-240min} concentrations of sitagliptin range(median) 3367.5–132645 (66998.3) ng/mL and 9.7 fold variation for gliclazide 196305-1905450 (585975) ng/mL.

Reduced renal function is associated with higher trough plasma levels of sitagliptin.

The association between baseline characteristics and plasma concentrations of sitagliptin and gliclazide are shown in *Table 3 and 4*. Renal function (eGFR) and age were both strongly associated with plasma trough levels of sitagliptin linear regression $\beta = -2.3$, 95%CI (-3.64, -0.99) $p = 0.001$ for age and $\beta = 3.9$, 95%CI (0.84 to 7), $p = 0.01$ for renal function respectively (*Figure 6b*). However in multivariable analysis including renal function and age, only renal function was independently associated: eGFR $\beta = -1.93$, 95%CI (-3.4, -0.41) $p = 0.01$, age $\beta = 1.8$, 95%CI (-1.6, 5.2), $p = 0.3$ respectively. These features were not associated with trough gliclazide level. Other clinical determinants including gender, BMI, weight and duration of disease were also not associated with trough plasma levels of

sitagliptin or gliclazide (*Table 3*). Similar results were seen when assessing total $AUC_{0-240min}$ drug concentrations (*Table 4*).

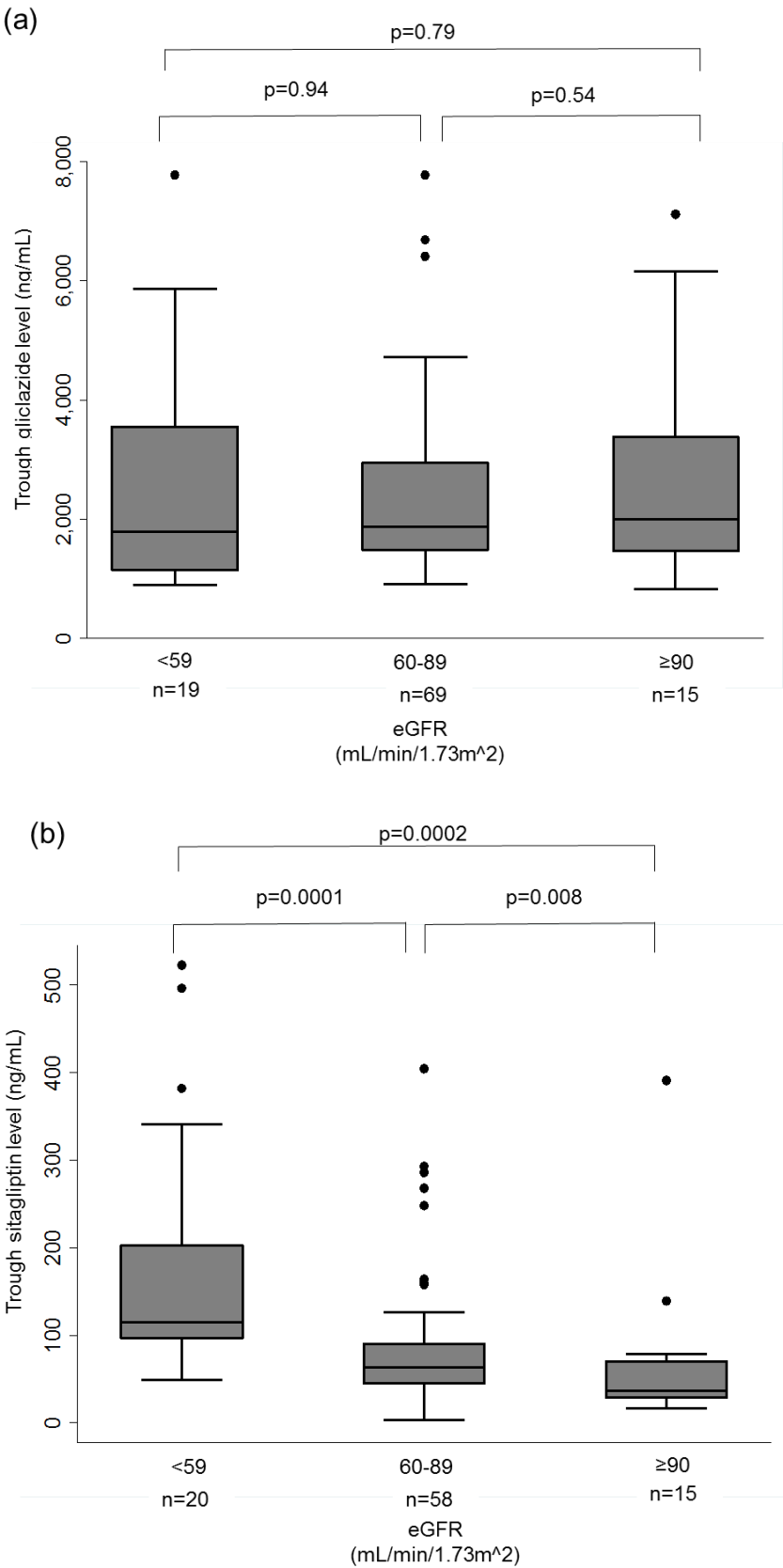


Figure 6 - box and whisker plot demonstrating relationship between trough levels of gliclazide and eGFR (a) and box and whisker plot demonstrating how decreasing glomerular filtration rate (according to CKD classification) increases trough levels of sitagliptin (b).

Trough Sitagliptin Level							Trough Gliclazide Level					
	(n)	β	SE	R squared	(95% CI)	p value	(n)	β	SE	R squared	(95% CI)	p value
eGFR	93	-2.3	0.67	0.12	-3.64, -0.99	0.001	93	-1.5	11	0.0002	-23.3, 20.3	0.89
Age	93	3.9	1.54	0.07	0.84, 7	0.01	94	-7.9	24.7	0.001	-56.1, 41.1	0.75
BMI	92	4.03	2.23	0.03	-0.4, 8.5	0.07	92	-23.4	32.3	0.006	-87.5, 40.7	0.47
Weight	92	0.93	0.67	0.02	-0.41, 2.27	0.17	93	-10.31	9.9	0.01	-30.1, 9.4	0.3
Gender	93	-11.42	23.4	0.003	-57.9, 35.1	0.63	94	-295	359	0.007	-1008, 419	0.42

Table 3- Association of trough drug levels of sitagliptin and gliclazide with clinical determinants, using univariate linear regression analysis.

Total AUC _{0-240min} Sitagliptin Level							Total AUC _{0-240min} Gliclazide Level					
	(n)	β	SE	R squared	(95% CI)	p value	(n)	β	SE	R squared	(95% CI)	p value
eGFR	58	-762.3	333	0.1	-1429, -95.3	0.03	58	1216	3685	0.002	-6168,8600	0.74
Age	58	251	725.6	0.002	-1203, 1705	0.73	58	-5476	7662	0.01	-20824, 9872	0.48
BMI	57	683	1047	0.01	-1416, 2782	0.52	57	5852	10768	0.01	-15727, 27430	0.59
Weight	57	-26.4	292.4	0.93	-613, 560	0.93	57	782.4	3002	0.001	-5233, 6798	0.8
Gender	58	7895	10938	0.009	-14016, 29807	0.47	58	-15116	116413	0.0003	-248329, 218087	0.9

Table 4- Association of total AUC₀₋₂₄₀ drug levels of sitagliptin and gliclazide with clinical determinants, using univariate linear regression analysis.

Trough plasma concentrations of sitagliptin and gliclazide are not associated with glycaemic response.

We found no evidence of a continuous association between trough plasma concentrations of sitagliptin and gliclazide and (baseline and treatment order adjusted) fasting glucose $\beta=0.0005$, 95%CI (-0.002, 0.003) $p=0.74$ and $\beta=-0.0006$, 95%CI (-0.0003, 0.0002) $p=0.58$ (Figures 7a and 7b respectively).

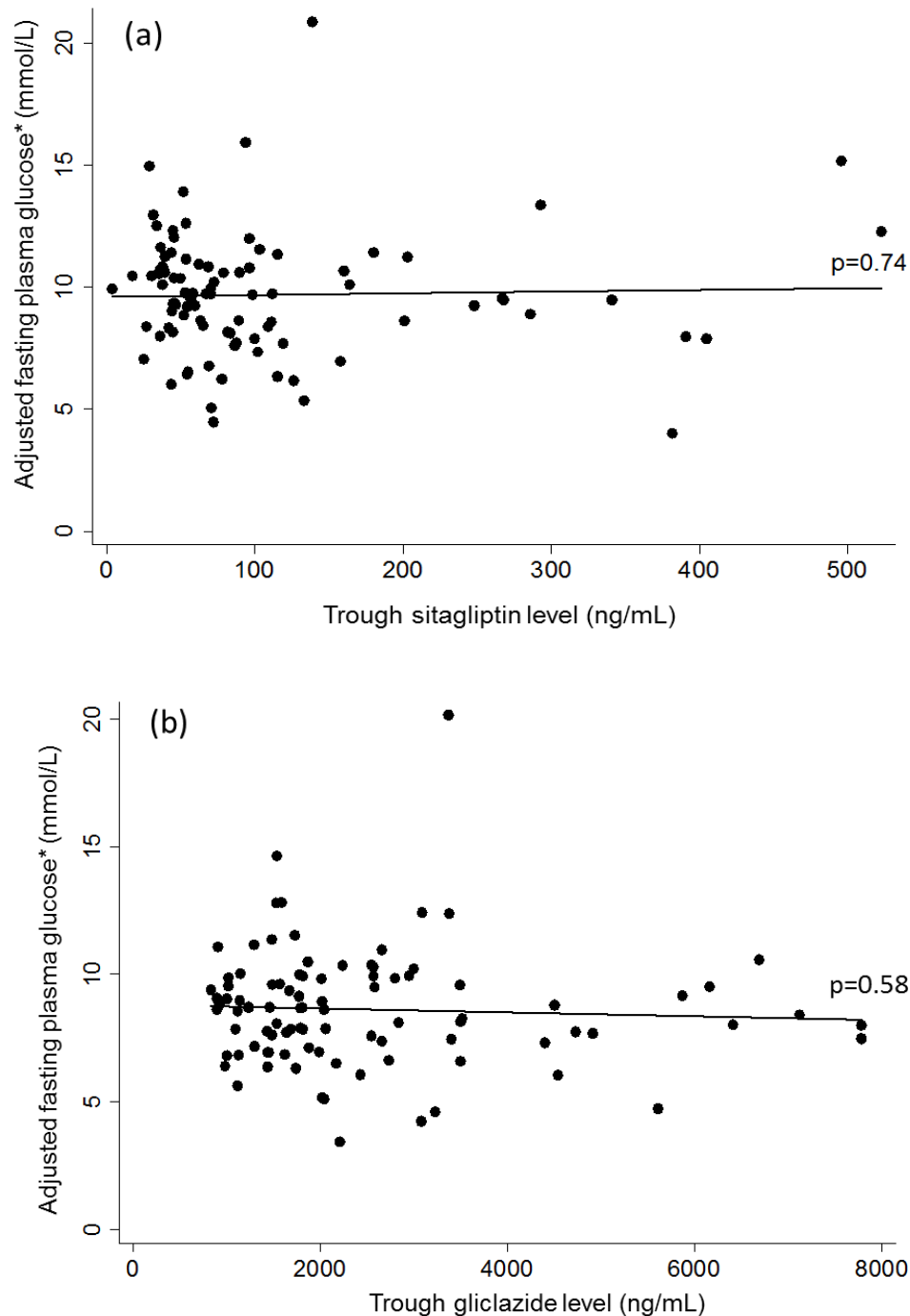


Figure 7- scatter plots showing the association between: fasting glucose levels and trough plasma drug levels of sitagliptin (a) and gliclazide (b) *all glucose outcomes have been adjusted to mean baseline glucose of 9.3mmol/L for fasting glucose outcomes and were also adjusted for drug order.

There was also no evidence of a relationship between trough drug levels and postprandial (incremental $AUC_{0-240min}$) glucose response using the same adjusted linear regression analysis (*Figure 8a and 8b*): $\beta = 0.18$, 95%CI (0.17, 0.14) $p=0.84$ for sitagliptin and $\beta = 0.15$, 95%CI (0.0004, 0.02) $p=0.06$ for gliclazide respectively. Similarly, we found no evidence of a continuous association between trough plasma drug levels and fructosamine response; $\beta = 0.04$, 95%CI (-0.06, 0.14) $p=0.44$ for sitagliptin and $\beta = -0.03$, 95%CI (-0.01, 0.002) $p=0.24$ for gliclazide (*Supplementary Table 3 and 4, see page 80*)

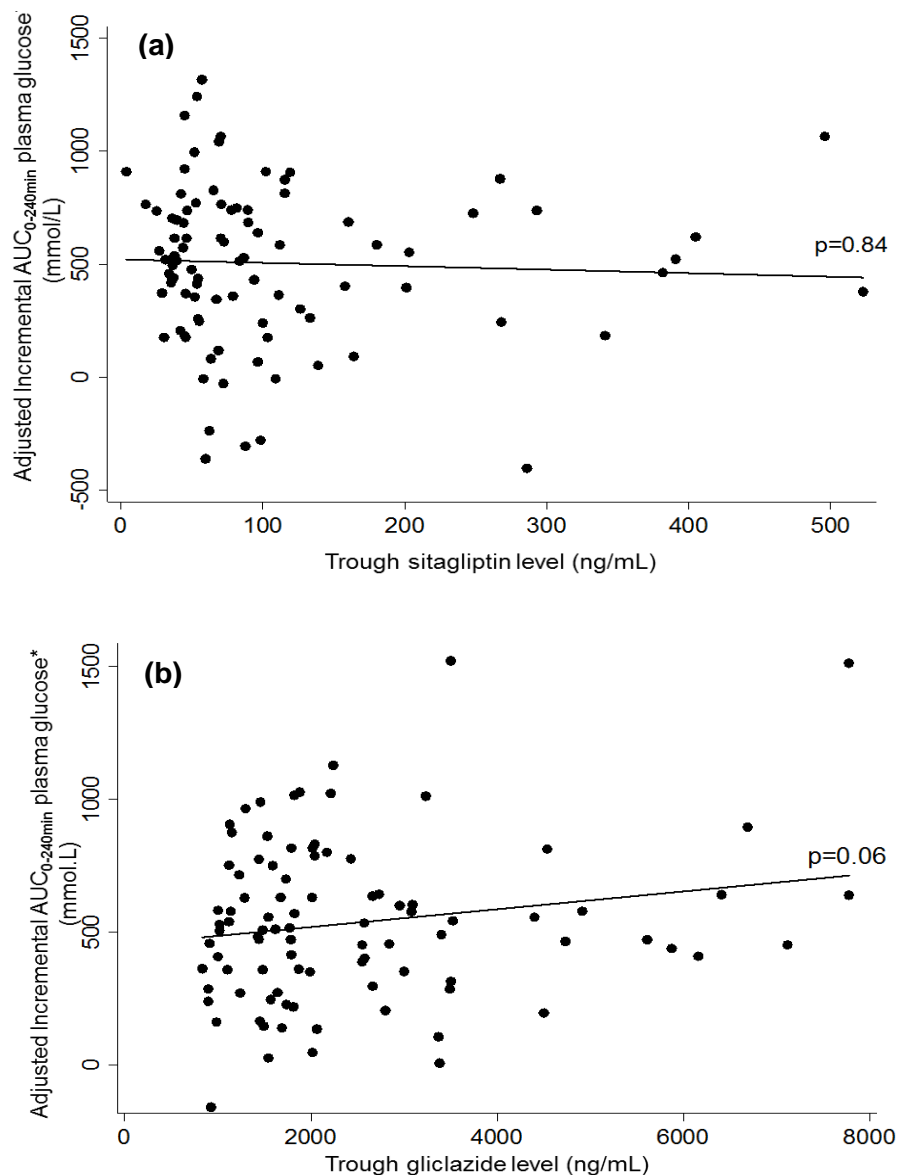


Figure 8- scatter plots showing the association between: incremental $AUC_{0-240min}$ glucose and trough plasma drug levels of sitagliptin (a) and gliclazide (b),*all glucose outcomes have been adjusted to mean baseline glucose of 417.4 for incremental $AUC_{0-240min}$. All glucose outcomes were also adjusted for drug order.

In categorical analysis that compared glycaemic response between those with the lowest trough drug levels (lowest quartile) to remainder, we found no association between low trough drug levels and baseline glucose and drug order adjusted fasting glucose levels for either agent: (sitagliptin: low mean adjusted glucose = 9.2 mmol/L, high mean adjusted glucose = 10.2 mmol/L. $p=0.28$ or gliclazide: low mean adjusted glucose = 8.7 mmol/L, high mean adjusted glucose = 7.8 mmol/L, $p=0.65$) or post meal glucose response (sitagliptin $p=0.16$ or gliclazide $p=0.4$) (*Table 5*).

Sitagliptin			
Outcome	Mean Low Group (n=24)	Mean High Group (n=69)	p value
Fasting Glucose	9.2	10.2	0.28
Incremental AUC _{0-240min} Glucose	492	619	0.16
Gliclazide			
Outcome	Mean Low Group (n=24)	Mean High Group (n=70)	p value
Fasting Glucose	8.7	7.8	0.65
Incremental AUC _{0-240min} Glucose	571	509	0.40

Table 5- : analysis performed using ANCOVA to determine if individuals within the 25th centile of trough drug levels have a difference in glycaemic response to glucose-lowering therapy compared to those with greater trough drug levels, adjusted for baseline glucose and drug order.

Total AUC_{0-240min} plasma concentrations of sitagliptin and gliclazide do not influence rise in postprandial glycaemic response to treatment

Similarly, as shown in *Figures 9a and 9b*, analysis of our dataset also revealed no association between total AUC_{0-240min} plasma drug concentrations and incremental AUC_{0-240min} plasma glucose: sitagliptin $\beta = -0.06$, 95%CI (-0.00002, 5.7×10^{-6}) $p=0.28$ and gliclazide $\beta = -0.09$, 95%CI (-7.81×10^{-7} , 1.02×10^{-6}), $p= 0.79$. Results were similar for total AUC₀₋₂₄₀ glucose and fructosamine (*Supplementary Table 3 and 4 respectively*).

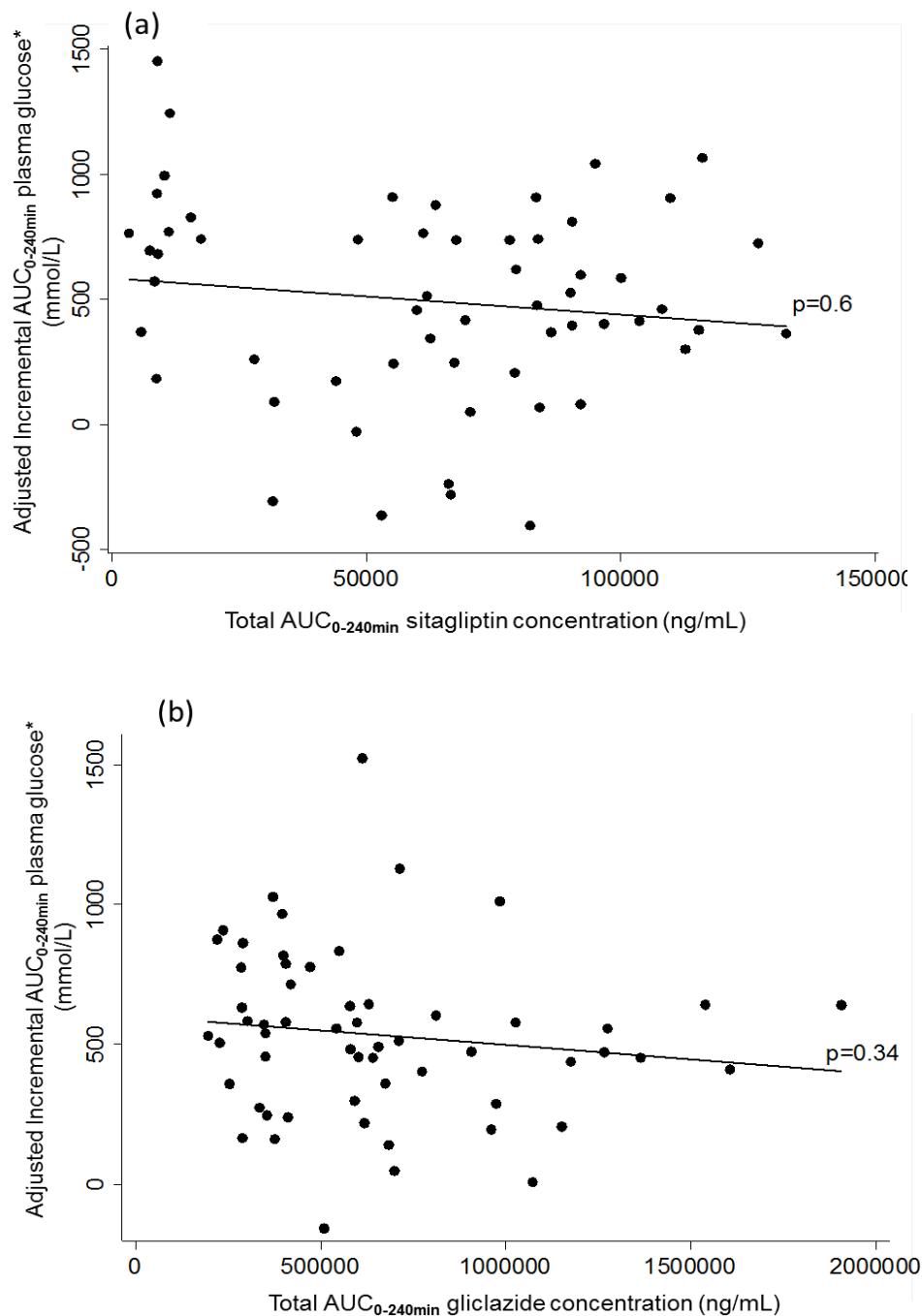


Figure 9- scatter plots showing the association between incremental AUC_{0-240min} plasma glucose and total AUC_{0-240min} drug levels of sitagliptin (a) and gliclazide (b) *all glucose outcomes have been adjusted to mean baseline glucose of 9.3mmol/L for fasting glucose outcomes and 417.4 for incremental AUC_{0-240min}. All glucose outcomes were also adjusted for drug order.

3.4 Discussion

Our results did not show an association between achieved plasma drug levels and glucose lowering response to sitagliptin and gliclazide, despite wide variations in achieved drug levels. We found that trough and total AUC_{0-240min} plasma drug concentrations of sitagliptin and gliclazide were highly variable despite all participants receiving a standard dose. Our results indicate that worsening renal function is associated with increased plasma levels of sitagliptin, which is consistent with current literature [1–3] and current prescribing guidelines. In contrast, plasma gliclazide levels did not alter with participant clinical characteristics.

Comparison with other studies

To our knowledge, this is the only study to examine the relationship between plasma drug levels and glycaemic response. Previous studies that aimed to determine doses of DPP-4 inhibitors have reported mean effects of DPP-4 inhibition and glucose response with dose variation, but have not reported variation in plasma drug concentrations or their effects. A crossover study by Herman et al. observed a similar glucose lowering response and incretin effect in patients with T2D who were given 25 mg or 200 mg of sitagliptin after 24 hours when compared to that measured after 2 hours [4]. Their results also report an association between a DPP-4 inhibition of $\geq 80\%$ (corresponding to a plasma concentration of 100 nM of sitagliptin) and a near to maximum reduction of glycaemia. These findings are further supported by a randomised controlled crossover trial conducted by Alba et al., who reported no significant differences in the percentage of DPP-4 inhibition and glucose lowering effects after 24 hours between sitagliptin dosages of 100 mg and 200 mg per day [5]. The lack of a dose-response relationship found in these studies is consistent with our findings that variation in sitagliptin levels do not substantially explain variation in glycaemic response. These findings may be explained by a threshold effect, where maximum efficacy (DPP-4 enzyme inhibition) and the corresponding reduction in glycaemia, is achieved at very low drug doses and plasma concentrations.

Our findings are also consistent with results from a recent meta-analysis of 31 randomised control trials which aimed to estimate the effects of sulphonylurea therapy on lowering HbA1c [6]. Analysis of dose-comparison trials for two sulphonylurea drugs, glimepiride and glipizide, failed to demonstrate that higher

doses significantly lowered HbA1c more than lower doses [7]. However our results are in contrast to previous pharmacogenetic studies that examined the role of genetic variations in cytochrome p450 enzymes, enzymes pivotal to sulphonylurea metabolism that are associated with drug levels and therapeutic response. One study investigated the effects of CYP2C9 genetic polymorphisms on the efficacy and pharmacokinetics of the sulphonylurea glimepiride in Japanese subjects with T2D. It reported AUC concentrations of glimepiride to be 2.5 folds higher and a greater reduction in HbA1c in individuals who were carriers of CYP2C9*1/*3 compared to carriers of the wild type [8]. This is further supported by analysis of retrospective data collated from the GoDarts study on 1073 incident sulphonylurea users (80% of which were treated with gliclazide only), which showed that individuals with two copies of loss-of-function CYP2C9 alleles had a 0.5% greater reduction in HbA1c and were 3.4 folds more likely to achieve their glycaemic targets compared to wild carriers [9]. Of note, a further study did not find a relationship between these variants and gliclazide levels in a Chinese population [10].

Strengths and Limitations

A key strength of this study is its design as a randomised control crossover trial; this is the optimal design for assessing individual factors that may influence variation in treatment response. The quality of the data is strengthened by its interventional trial setting, with a robust assessment of glycaemic response through mixed meal tolerance tests, and robust trough and area under the curve assessment of drug levels over several time points. Other strengths include the novelty of our research, as previously mentioned, this is the first study to identify that plasma drug levels are not associated with therapeutic response in second line diabetic agents, an area where research is limited.

This study is limited by its modest sample size and short duration, which precludes assessment of HbA1c response. While our results show that drug levels are not a major driver of the substantial variation in short term drug response, our sample size is insufficient to exclude small effects or potential threshold effects at very low plasma drug levels. A further important limitation is that as baseline samples were only collected up to 2 hours, (instead of 3 and 4 hours), meaning all analysis of incremental AUC_{0-240min} glucose response had to

be adjusted for the 2 hour incremental AUC glucose at baseline instead of the full 4 hours. Despite this, the full 4 hour mixed meal tests these values were highly correlated ($r = 0.82$ and 0.88) for tests conducted after administration of gliclazide and sitagliptin respectively. Lastly, our data did not capture peak gliclazide concentrations, meaning our results do not provide key pharmacokinetic data for gliclazide such as T_{max} and C_{max} . Previous studies have been successful in capturing peak concentrations of gliclazide over a 4 hour period post oral glucose tolerance test (OGTT) [11], however the constituents of the MMTT in this study may have decreased the gliclazide absorption rate.

Clinical and research implications

Our results support current guidance to reduce doses of sitagliptin in patients diagnosed with chronic kidney disease, as they show that increased plasma concentrations of sitagliptin are associated with worsening renal function. Our results also support current practice that, with the exception of sitagliptin and renal function, recommended doses of sitagliptin and gliclazide should not differ in patients with different clinical features, such as body weight.

Our findings have potential implications in integrating a stratified approach to T2D treatment. We have shown that plasma drug levels of the two most commonly prescribed second line agents do not substantially influence therapeutic response to treatment. It is therefore likely that mechanisms other than plasma drug levels underpin the variation in therapeutic response. For clinical practice, our results suggest that increasing the dose of these medications in individuals with an apparent poor glycaemic response is unlikely to be associated with major glycaemic benefit. Consistent with this, there is published evidence that increasing sulfonylurea dose within the clinical dose range may reduce effectiveness, or at best result in no improvement in glycaemic response [12] .

Our findings also have implications on precision medicine research in diabetes; they indicate that conducting precision medicine studies to measure plasma drug levels on a large scale is unlikely to significantly further to our understanding of the mechanisms or effective predictors of treatment response. Our results also suggest that characteristics and biomarkers associated with drug levels will not

greatly explain variation in response to these medications for the majority of patients.

3.5 Conclusion

Understanding the mechanisms underpinning this variation and identifying robust predictors of response may allow the integration of precision medicine to T2D treatment [12]. This approach encourages therapy choice based on what most likely to be effective or tolerated for a group or subgroup of patients based on their characteristics, thus minimising adverse effects and maximising therapeutic benefit [13]. Lastly, understanding reasons for variation in drug response could aid in dose selection and assist the design of new medications with lower non-response rates.

In conclusion our findings show that variation in plasma drug levels do not substantially contribute to variation in glucose lowering response to gliclazide and sitagliptin therapy. It is therefore likely that variation in therapeutic response to these agents is driven by other factors, which should be the focus of future research.

3.6 Supplementary Tables

Response	Effect (95%CI)	p	n
Treatment effect	1.24 (0.82,1.65)	<0.001	95
Period Effect	-0.35 (-0.77,0.06)	0.1	95
Carryover Effect	-1.2 (-3.07,0.67)	0.2	95

Change on treatment	Effect (95%CI)	p	n
Treatment effect	1.47 (0.86,2.07)	<0.001	90
Period Effect	0.21 (-0.39,0.82)	0.49	90
Carryover Effect	0.71 (-0.44,1.86)	0.22	90

Supplementary Table 1 - *treatment, period and carry over effects on fasting glucose.*

Response	Effect (95%CI)	p	n
Treatment effect	23.75 (14.63,32.87)	<0.001	104
Period Effect	-18.5 (-27.62,-9.38)	<0.001	104
Carryover Effect	8.54 (-50.86,67.94)	0.78	104

Change on treatment	Effect(95%CI)	p	n
Treatment effect	44.56 (31.4,57.72)	<0.001	103
Period Effect	5.79 (-7.37,18.95)	0.38	103
Carryover Effect	-8.68 (-27.55,10.2)	0.36	103

Supplementary Table 2- *treatment period and carry over effects on fructosamine.*

Sitagliptin						Gliclazide					
(n)	β	SE	R squared	(95% CI)	p value	(n)	β	SE	R squared	(95% CI)	p value
Trough drug level	93	-0.02	0.08	0.37	-0.17, 0.14	0.84	93	0.01	0.004	-0.0004, 0.02	0.06
Total drug AUC _{0-240min}	58	-0.001	0.001	0.46	-0.003, 0.002	0.6	58	-0.0001	0.0001	-0.0002, 0.0001	0.34
Peak drug levels	58	-0.0002	0.0003	0.46	-0.001, 0.0005	0.6	58	-0.00002	-0.00002	-0.0001, 0.00002	0.34

Supplementary Table 3- Association of drug levels (trough, total AUC_{0-240min} and peak) of sitagliptin and gliclazide with incremental AUC_{0-240min} post meal glucose change from baseline. Analysis performed using linear regression, adjusted for baseline glucose and drug order.

Fructosamine (sitagliptin)						Fructosamine (gliclazide)					
	(n)	β	SE	(95% CI)	p value	(n)	β	SE	(95% CI)	p value	
Trough drug level	93	0.04	0.05	-0.06, 0.14	0.44	93	-0.003	0.002	-0.01, 0.002	0.24	
TotalAUC _{0-240min} drug level	58	0.0002	0.0002	-0.0002, 0.001	0.38	58	-4.71x10 ⁻⁰⁶	0.00001	-0.00003, 0.00002	0.73	

Supplementary Table 4- Association of drug levels (Trough, Total AUC_{0-240min}) of sitagliptin and gliclazide with fructosamine. Analysis performed using linear regression, adjusted for baseline glucose and drug order.

3.7 References

1. Arjona Ferreira JC, Marre M, Barzilai N, et al (2013) Efficacy and safety of sitagliptin versus glipizide in patients with type 2 diabetes and moderate-to-severe chronic renal insufficiency. *Diabetes Care* 36:1067–1073 . doi: 10.2337/dc12-1365
2. Arjona Ferreira JC, Corry D, Mogensen CE, et al (2013) Efficacy and safety of sitagliptin in patients with type 2 diabetes and ESRD receiving dialysis: a 54-week randomized trial. *Am J Kidney Dis* 61:579–587 . doi: 10.1053/j.ajkd.2012.11.043
3. Bergman AJ, Cote J, Yi B, et al (2007) Effect of renal insufficiency on the pharmacokinetics of sitagliptin, a dipeptidyl peptidase-4 inhibitor. *Diabetes Care* 30:1862–1864 . doi: 10.2337/dc06-2545
4. Herman GA, Bergman A, Stevens C, et al (2006) Effect of single oral doses of sitagliptin, a dipeptidyl peptidase-4 inhibitor, on incretin and plasma glucose levels after an oral glucose tolerance test in patients with type 2 diabetes. *J Clin Endocrinol Metab* 91:4612–4619 . doi: 10.1210/jc.2006-1009
5. Alba M, Sheng D, Guan Y, et al (2009) Sitagliptin 100 mg daily effect on DPP-4 inhibition and compound-specific glycemic improvement. *Curr Med Res Opin* 25:2507–2514 . doi: 10.1185/03007990903209514
6. Hirst JA, Farmer AJ, Dyar A, et al (2013) Estimating the effect of sulfonylurea on HbA1c in diabetes: a systematic review and meta-analysis. *Diabetologia* 56:973–984 . doi: 10.1007/s00125-013-2856-6
7. Simonson DC, Kourides IA, Feinglos M, et al (1997) Efficacy, safety, and dose-response characteristics of glipizide gastrointestinal therapeutic system on glycemic control and insulin secretion in NIDDM. Results of two multicenter, randomized, placebo-controlled clinical trials. The Glipizide Gastrointestinal Therapeutic System Study Group. *Diabetes Care* 20:597–606

8. Suzuki K, Yanagawa T, Shibasaki T, et al (2006) Effect of CYP2C9 genetic polymorphisms on the efficacy and pharmacokinetics of glimepiride in subjects with type 2 diabetes. *Diabetes Res Clin Pract* 72:148–154 . doi: 10.1016/j.diabres.2005.09.019
9. Zhou K, Donnelly L, Burch L, et al (2010) Loss-of-function CYP2C9 variants improve therapeutic response to sulfonylureas in type 2 diabetes: a Go-DARTS study. *Clin Pharmacol Ther* 87:52–56 . doi: 10.1038/clpt.2009.176
10. Zhang Y, Si D, Chen X, et al (2007) Influence of CYP2C9 and CYP2C19 genetic polymorphisms on pharmacokinetics of gliclazide MR in Chinese subjects. *Br J Clin Pharmacol* 64:67–74 . doi: 10.1111/j.1365-2125.2007.02846.x
11. Davis TME, Daly F, Walsh JP, et al (2000) Pharmacokinetics and pharmacodynamics of gliclazide in Caucasians and Australian Aborigines with type 2 diabetes. *Br J Clin Pharmacol* 49:223–230 . doi: 10.1046/j.1365-2125.2000.00162.x
12. Garber AJ (2000) Using dose-response characteristics of therapeutic agents for treatment decisions in type 2 diabetes. *Diabetes, Obesity and Metabolism* 2:139–147 . doi: 10.1046/j.1463-1326.2000.00054.x
13. Hattersley AT, Patel KA (2017) Precision diabetes: learning from monogenic diabetes. *Diabetologia* 60:769–777 . doi: 10.1007/s00125-017-4226-2

Chapter 4

Chapter 4: can variation in adherence explain the variation in glycaemic response to sitagliptin and gliclazide therapy?

4.1 Introduction

A key aim of precision diabetes is to identify predictors of therapeutic response to glucose lowering medication. This will only benefit clinical practice if it provides evidence that non-response to one medicine may be associated with good response to another medicine with an alternative mechanism of action, rather than a lack of response to hypoglycaemic medication. One of the first steps in identifying predictors of response is understanding how much non-response to medication is attributed to non-adherence rather than an intrinsic lack of response.

Adherence to prescribed glucose lowering therapy has frequently been identified as a major factor in achieving adequate glycaemic control and subsequent mortality rates amongst the T2D cohort[1]. Analysis of primary care records, adjusted for confounding factors, has shown that non-adherence to prescribed medications and clinic non-attendance were independent risk factors for death among individuals with type 2 diabetes [2].

Few studies have examined the relationship between diabetes medication adherence and glucose-lowering response; with the majority of studies limited by their small sample size or specific focus on metformin and sulphonylurea therapy[3].

If measurable variations in medication adherence is associated with the variation in glucose-lowering response to T2D therapy, then measuring adherence provides a mechanism to increase precision in future studies of treatment stratification in T2D. Therefore we aimed to investigate whether variation in rates of adherence to sitagliptin 100mg OD and gliclazide 80mg OD in a randomised controlled trial setting are a major cause of variation in glucose lowering response to these therapies.

4.2 Methods

4.2.1 Study design

We assessed the relationship between adherence to gliclazide and sitagliptin and glycaemic response in the MASTERMIND pilot study, an open label, randomized, two arm crossover trial conducted in the south west of England and Dundee, Scotland (a detailed overview of the study design can be found in *Chapter 2*)

4.2.2 Measurement of Adherence

MEMS Caps

Baseline medication adherence data was obtained through self-reported compliance, a validated medication adherence score and practice prescription records. During each 4 week treatment period, participants took a single dose of sitagliptin (100mg) or gliclazide (80mg) once daily in the morning. All study medication was given in containers with Medication Event Monitoring System (MEMS) caps that record opening times to assist assessment of medication adherence (for MEMS caps are the gold standard), with remaining tablets counted at study visits.

Mean Possession Ratio

Participant adherence to study medication was calculated using a Mean Possession Ratio (MPR), which is the sum of the days where medication is taken as prescribed in a particular time period, divided by the number of days in that time period, expressed as a percentage. Further details of calculating MPR can be cited[4, 5].

4.2.3 Glycaemic response.

Fructosamine, a 2-3 week measure of glycaemia, is a key marker of therapeutic response for this analysis. This is because study medication was administered directly prior to the MMTT assessment and therefore may skew the glycaemic effects of poor adherence over the 4-week treatment period, particularly when assessing short term glycaemic response (FPG and PPG).

4.2.4 Statistical Analysis

Initially, we explored at the prevalence of non-adherence defined by a MPR <80%. We then assessed the relationship between MPR as a continuous covariate and response, followed by a secondary categorical analysis to compare glycaemic responses to sitagliptin and gliclazide between participants who were adherent and non-adherent.

Assessing the continuous relationship between adherence and glycaemic response

We assessed the continuous relationship between medication adherence and glycaemic outcomes (on therapy fructosamine, fasting glucose, total AUC_{0-240min} and incremental AUC_{0-240min} (calculated using the trapezoid rule)) using linear regression. All linear regression models were adjusted for the baseline glucose measurement at initial MMTT (see *Chapter 3*).

Comparison of glycaemic response in adherent participants who are adherent vs non-adherent.

Participants were defined as adherent if their Medication Possession Ratio (MPR) $\geq 80\%$ and non-adherent if MPR <80%. This cut-off is based on robust evidence that demonstrates, reduced treatment response and increased mortality/hospital admissions for patients who had medication adherence rates <80% [3, 6].

We assessed whether participants who were non-adherent (MPR<80%) have reduced glycaemic response compared to individuals who were adherent (MPR $\geq 80\%$) via a secondary categorical analysis comparing glycaemic response in adherent and non-adherent participants, using ANCOVA with the baseline measurement of the glycaemic endpoint and drug order as covariates.

4.3 Results

Normally distributed data is expressed as mean(SD) and non-normal distributed data is given as median(IQR).

Participants

Of 127 participants who had completed the study per protocol, adherence data was collected 110 participants and 112 participants for sitagliptin and gliclazide respectively.

The majority of study participants were adherent ≥ 80 percent

The number of participants who were adherent n(%) to sitagliptin was 103(93.6) and to gliclazide was 109(97.9) (*Figure 10*). The median(IQR) adherence for was 100(96.4-100) for sitagliptin and 100 (96.4-100) for gliclazide.

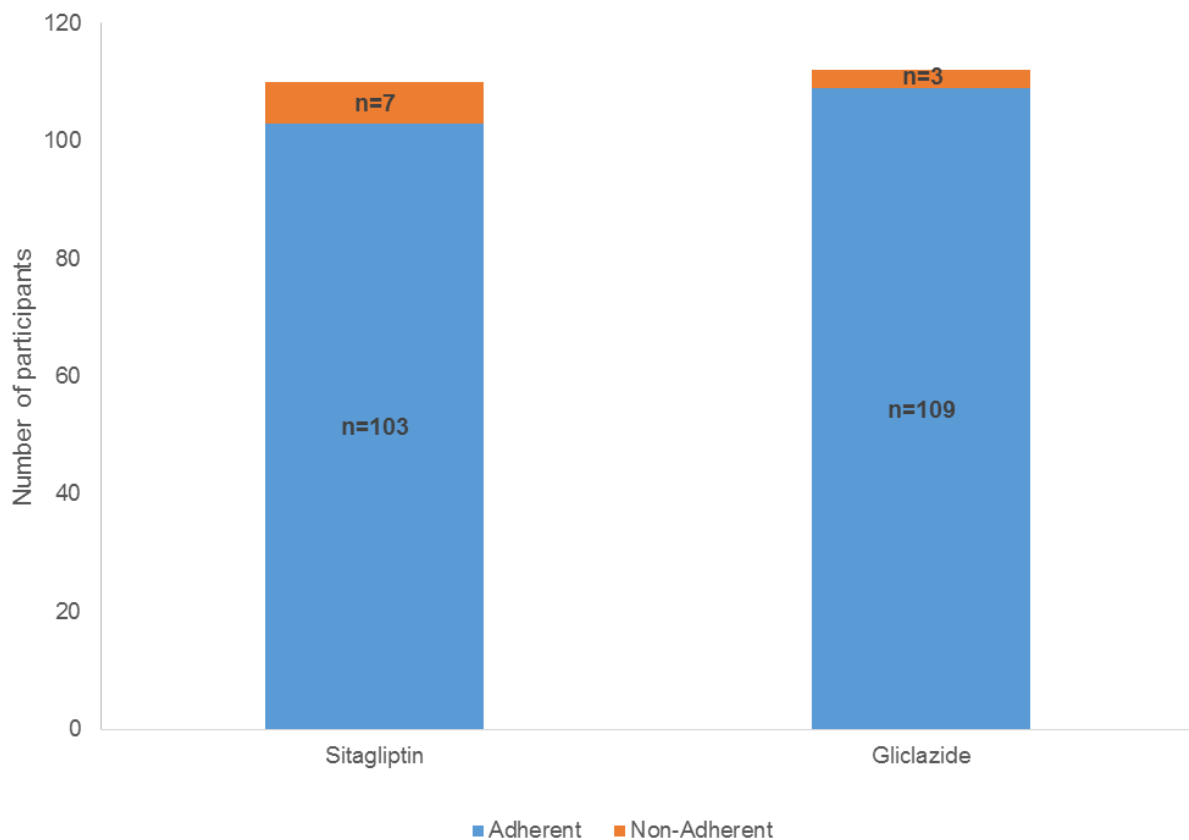


Figure 10 Bar chart representing number of individuals who were adherent to sitagliptin (left) and gliclazide (right)

There is no continuous association between adherence and glucose-lowering response to therapy.

Linear regression analysis showed no evidence of continuous association between medication adherence adjusted fructosamine response: $\beta(95\%CI) = -0.32 \text{ umol/L/\%} (-1.12, 0.47)$, $p=0.42$ and $-0.05 \text{ umol/L/\%} (-0.67, 0.58)$ $p=0.88$ for sitagliptin and gliclazide respectively

Our findings also show no evidence of a continuous association between adjusted fasting glycaemic response and adherence to sitagliptin or to gliclazide (*Table 6*): linear regression: $\beta(95\%CI)$ was $-0.02 \text{ mmol/L/\%} (-0.04, 0.01)$, $p=0.17$ and $0.01 \text{ mmol/L/\%} (-0.02, 0.03)$ respectively.

When investigating the relationship between adherence to sitagliptin and total $AUC_{0-240\text{min}}$ glucose response, a significant association was noted: $\beta(95\%CI)$ $0.01\text{-}0.02 \text{ mmol/L/\%} (-0.02, 0.03)$, $p=0.049$ after adjusting for baseline glucose and drug order. Contrastingly, we found no evidence of a relationship between adherence to gliclazide therapy and total $AUC_{0-240\text{min}}$ glucose concentrations.

Similarly, we found no evidence continuous association between medication adherence and rise in post meal glucose levels: $\beta(95\%CI)$ for sitagliptin was $-2.76 \text{ mmol/L/min/\%} (-7.69, 2.17)$, $p=0.27$ and $0.71 \text{ mmol/L/min/\%} (-3.21, 4.63)$ $p=0.72$ for gliclazide.

Sitagliptin							Gliclazide					
Glycaemic outcome	(n)	β	SE	(95% CI)	t	p value	(n)	β	SE	(95% CI)	t	p value
Fasting Glucose	110	-0.02	0.02	-0.04, 0.01	0.17	0.17	112	0.01	0.01	-0.02, 0.03	0.69	0.49
Total AUC _{0-240min} glucose	110	-7.8	3.9	-15.6, -0.03	-1.99	0.049	112	2.6	3.31	-3.96, 9.17	0.79	0.43
Incremental AUC _{0-240min} glucose	110	-2.76	2.49	-7.69, 2.17	-1.11	0.27	112	0.71	1.98	-3.21, 4.63	0.36	0.72
Fructosamine	110	-0.32	0.4	-1.12, 0.47	-0.8	0.42	112	-0.05	0.31	-0.67, 0.58	-0.15	0.88

Table 6 - continuous association between adherence and glycaemic response (fasting, total AUC_{0-240min}, incremental AUC_{0-240min} and fructosamine. Analysis was performed using linear regression with adjustment for baseline glucose and drug order.

There was no difference in glycaemic response between adherent and non-adherent participants to study medication.

As shown in *Table 7* categorical analysis revealed no significant difference in fructosamine response between individuals who were non-adherent vs adherent. For sitagliptin, mean fructosamine response was 377 $\mu\text{mol/L}$ vs 382 $\mu\text{mol/L}$, $p=0.6$ and for gliclazide was 338 $\mu\text{mol/L}$ vs 359 $\mu\text{mol/L}$, $p=0.98$ for nonadherent vs adherent individuals respectively

We also found no evidence in a significant difference in fasting glycaemic response between non-adherent vs adherent participants to either study therapy. Mean fasting glucose for non-adherent vs adherent participants was 9.97 mmol/L vs 9.7 mmol/L , $p=0.9$ for sitagliptin and 7.13 mmol/L vs 8.52 mmol/L , $p=0.29$ for gliclazide.

We found similar results when comparing differences incremental $\text{AUC}_{0-240\text{min}}$ responses between non-adherent vs adherent individuals: the mean rise in post-meal (incremental $\text{AUC}_{0-240\text{min}}$) glucose was 430 vs 568 mmol/L/min $p=0.56$ for sitagliptin and 474 vs 589 mmol/L/min , $p=0.64$ for gliclazide respectively.

Outcome (sitagliptin)	Adherent (≥80%)	Non-adherent (<80%)	p value
Mean fasting glucose	9.7	9.97	0.9
Mean total AUC _{0-240min} glucose	2895	2832	0.98
Mean incremental AUC _{0-240min} glucose	568	430	0.56
Fructosamine	359	338	0.98

Outcome (gliclazide)	Adherent (≥80%)	Non-adherent (<80%)	p value
Mean fasting glucose	8.52	7.13	0.29
Mean total AUC _{0-240min} glucose	2635	2194	0.25
Mean incremental AUC _{0-240min} glucose	589	474	0.64
Fructosamine	382	377	0.6

Table 7- analysis performed using ANCOVA to determine if individuals who were adherent have a difference in glycaemic response to glucose-lowering therapy compared to those who were not adherent. Results are shown at the means of baseline glucose and drug order.

4.4 Discussion

Our results show that the majority of participants were adherent to the study medication in this trial setting. Consistent with the very low rates of observed non-adherence, variation in adherence was largely not associated with glucose-lowering response to treatment, however a continuous association was found noted between total AUC_{0-240min} glucose levels and adherence to sitagliptin. Lastly, we found no evidence of a difference in glycaemic response between individuals who were adherent and non-adherence. Our findings must be interpreted in the context of the small cohort, with limited power to detect differences in response between those with good and poor adherence.

Comparison with other studies

In support of our findings, there is strong evidence to suggest that adherence to sitagliptin therapy is associated with glycaemic control in patients with T2D. One study investigated the relationship between adherence to metformin and sitagliptin and glycaemic control in 677 patients with T2D in southwest Michigan. Results revealed a significant association between a 10% increase in non-adherence and a 0.14%(1.5mmol/mol) increase in HbA1c [7]. In contrast to our findings is evidence from a larger study conducted of 1,668 patients in South Carolina that investigated adherence to metformin and sulphonylurea therapy and glycaemic response. It reported that the mean MPR of patients who reached a target HbA1c of 53mmol/mol (7.0%) was 81% (compared to a mean MPR of 72% in individuals who did not reach the target) [8], thus indicating an association between adherence to sulphonylurea therapy and glycaemic response.

A recent retrospective cohort analysis obtained from large clinical practice electronic databases in Scotland and England: GoDARTS (Scotland) and Clinical Practice Research Datalink, (CPRD) (England) also contest our findings. This study investigated whether adherence to glucose-lowering therapy was associated with HbA1c levels after one year of commencing treatment. Analysis of 32,634 and 2,284 found that 13% and 15% of participants from GoDarts and CPRD respectively were non-adherent (non-adherence was defined by an MPR <80% calculated using prescription record data). A smaller reduction in 1-year HbA1c was noted when non-adherent patients were compared with the adherent cohort: 0.46% (5.0 mmol/mol) for GoDarts and 0.4%(4.4 mmol/mol) for CPRD respectively. Interestingly, differences in glycaemic response between non-

adherent and adherent groups varied according to the choice of hypoglycaemic therapy ranging from 0.38% (4.1 mmol/L to 0.75% (8.2 mmol/L)) however, reduced rates of adherence were consistently associated with a lower reduction in HbA1c [3]. A 2018 retrospective cohort analysis investigating medication adherence and diabetes outcomes also using data from CPRD further support these findings[9].

Strengths and Limitations

Strengths of this analysis include by the randomised control study design from which the data was obtained and the robust measurement of adherence using MEMS caps (the gold standard for measuring medication adherence) [10]. Despite this, our findings have several key limitations. Medication hoarding is a recognised concern in clinical practice [11] and although MEMS caps containers provide data on the number of times participants opened their medication containers, it cannot confirm whether participants actually took their medication. Furthermore, as participants were aware that they were partaking in a clinical trial where their medication administration was monitored, their medication-taking behaviour may have been altered. This challenges the clinical implications of our findings as they may not represent real life behaviour. A further key limitation is the small sample size of the cohort; the combination of low non-adherence rates and modest study size mean meaning our analysis has low power to detect effects of non-adherence on glycaemic response. Lastly, as medication was administered on visit days directly prior to the MMTT, the glycaemic consequences of non-adherence to study medication over the 4 week treatment period may have been negated, particularly as only short term glycaemic response was assessed. Therefore, to overcome this limitation, we have placed more emphasis on fructosamine response, which is a marker of glycaemia over 2-3 weeks.

Implications for future research

Our findings have implications for future research in precision diabetes; we have shown that adherence to two commonly used glucose-lowering agents does not substantially explain the variation in therapeutic response. If measurable differences in adherence explains the variation in glucose-lowering response to

diabetes therapy, then this would provide a mechanism to increase both precision and power in future studies in stratified diabetes.

If adherence to medication predicts glycaemic response, as suggested by current literature then the next focus of precision medicine should be to identify and address barriers to adherence. Such barriers arise from a multitude of complexities including medication tolerability, often dictated by side effects such as hypoglycaemia, weight gain and gastrointestinal disturbances. Additional barriers include polypharmacy, limited patient education, complexity of medication regimes and cognitive impairment (common in elderly patients). [12] Designing medications that reduce the frequency of administration, fixed-dose combinations is likely to aid in overcoming these barriers and concordance primarily by reducing pill burden [12].

Causes underlying patient non-adherence may alter during the course of the disease, however reports suggest that medication adherence is most likely to decrease in the first six months of commencing therapy [13]. It has been reported that patients with T2D who demonstrate poor adherence when commencing new oral hypoglycaemic agents are more likely to have a higher HbA1c and a delay in treatment intensification [13]. These findings have implications for treatment escalation and commencing de-novo therapy and suggest that if patients are adherent during the initial stages of commencing therapy then this may predict future glycaemic response.

4.5 Conclusion

We have shown that in a trial setting, observed differences in medication adherence to sitagliptin and gliclazide do not substantially explain the variation in glucose-lowering response therapy. As our sample size is underpowered to confidently detect true effects, our analysis should be repeated in a larger dataset that assess at glycaemic response over a longer time period (this will help to validate our findings using HbA1c response). If adherence to diabetes medications is found to implicate therapeutic response, then collaboration between researchers, clinicians and patients may help in identifying ways to effectively overcome barriers to adherence.

4.6 References

1. Simpson SH, Eurich DT, Majumdar SR, et al (2006) A meta-analysis of the association between adherence to drug therapy and mortality. *BMJ* 333:15 . doi: 10.1136/bmj.38875.675486.55
2. Currie CJ, Peyrot M, Morgan CL, et al (2012) The impact of treatment noncompliance on mortality in people with type 2 diabetes. *Diabetes Care* 35:1279–1284 . doi: 10.2337/dc11-1277
3. Farmer AJ, Rodgers LR, Lonergan M, et al (2016) Adherence to Oral Glucose-Lowering Therapies and Associations With 1-Year HbA1c: A Retrospective Cohort Analysis in a Large Primary Care Database. *Diabetes Care* 39:258–263 . doi: 10.2337/dc15-1194
4. Kozma CM, Dickson M, Phillips AL, Meletiche DM (2013) Medication possession ratio: implications of using fixed and variable observation periods in assessing adherence with disease-modifying drugs in patients with multiple sclerosis. *Patient Prefer Adherence* 7:509–516 . doi: 10.2147/PPA.S40736
5. Sperber CM, Samarasinghe SR, Lomax GP (2017) An upper and lower bound of the Medication Possession Ratio. *Patient Prefer Adherence* 11:1469–1478 . doi: 10.2147/PPA.S136890
6. Karve S, Cleves MA, Helm M, et al (2009) Good and poor adherence: optimal cut-point for adherence measures using administrative claims data. *Current Medical Research and Opinion* 25:2303–2310 . doi: 10.1185/03007990903126833
7. Pladevall M, Williams LK, Potts LA, et al (2004) Clinical outcomes and adherence to medications measured by claims data in patients with diabetes. *Diabetes Care* 27:2800–2805
8. Lawrence DB, Ragucci KR, Long LB, et al (2006) Relationship of oral antihyperglycemic (sulfonylurea or metformin) medication adherence and hemoglobin A1c goal attainment for HMO patients enrolled in a diabetes disease management program. *J Manag Care Pharm* 12:466–471 . doi: 10.18553/jmcp.2006.12.6.466

9. Gordon J, McEwan P, Idris I, et al (2018) Treatment choice, medication adherence and glycemic efficacy in people with type 2 diabetes: a UK clinical practice database study. *BMJ Open Diab Res Care* 6: . doi: 10.1136/bmjdr-2018-000512
10. Cohen HW, Shmukler C, Ullman R, et al (2010) Measurements of medication adherence in diabetic patients with poorly controlled HbA(1c). *Diabet Med* 27:210–216 . doi: 10.1111/j.1464-5491.2009.02898.x
11. Alsalman AJ, Smith WR (2013) Expanding the Framework of Assessing Adherence and Medication-Taking Behavior. *Journal of Pain & Palliative Care Pharmacotherapy* 27:114–124 . doi: 10.3109/15360288.2013.765532
12. Han S, Iglay K, Davies MJ, et al (2012) Glycemic effectiveness and medication adherence with fixed-dose combination or coadministered dual therapy of antihyperglycemic regimens: a meta-analysis. *Curr Med Res Opin* 28:969–977 . doi: 10.1185/03007995.2012.684045
13. Grant R, Adams AS, Trinacty CM, et al (2007) Relationship between patient medication adherence and subsequent clinical inertia in type 2 diabetes glycemic management. *Diabetes Care* 30:807–812 . doi: 10.2337/dc06-2170

Chapter 5

Chapter 5: measurable differences in weight and physical activity do not explain variation in response to sitagliptin or gliclazide therapy.

5.1 Introduction

Lifestyle interventions including weight loss and physical activity are at the cornerstone of T2D management [1]. Meta-analyses have demonstrated that structured aerobic exercise and resistance training have beneficial effects in reducing hyperglycaemia in T2D [2–4] however the studies included in these analyses are limited by their short duration and small sample size. Evidence from several robust interventional trials show that real-world physical activity advice in the absence of dietary interventions are insufficient to effectively lower HbA1c[2, 5, 6].

The influence of lifestyle factors on glucose-lowering response to diabetes medication remains unclear; with evidence from randomised controlled trials and post-hoc analyses yielding conflicting results[7–10]. As lifestyle advice (weight loss and physical activity) and oral diabetes therapies are prescribed concomitantly and independently lower blood glucose levels, it is possible that variation in physical activity may explain some of the observed variation in response to glucose-lowering therapies. We therefore aimed to investigate whether measurable differences in body weight physical activity can explain the variation in therapeutic response to sitagliptin and gliclazide therapy by analysing weight and accelerometer data from the MASTERMIND randomised control crossover trial (*Chapter 2*).

5.2 Methods

Assessment of weight

At visit 1 participants had measurements of their weight(kg), height(cm) and waist circumference(cm) recorded to obtain baseline data, with repeated measurements at each study visit.

Measurement and categorisation of physical activity

Physical activity was measured using a Geneactiv triaxial accelerometer. Participants' wore their accelerometers for 7 consecutive days (on their wrists) prior to on treatment visits (visit 4 and visit 6). Physical activity was classified as Light Physical Activity (LPA) or Moderate-to Vigorous Physical Activity (MVPA), depending on their mean acceleration in a 5s-epoch (ENMO). For LPA and MVPA this needed to be at or above 45.2 mg and 134.4mg respectively. The average daily minutes of physical activity (LPA or MVPA) for each treatment was calculated based on the 7 day recording. Calculation of LPA and MVPA was undertaken by Dr Lisa Philips, Lecturer in Physical activity Health Sport and Health Sciences, University of Exeter.

Statistical analysis

Weight and accelerometer data were assessed visually for their distribution, with non-normally distributed data expressed as median(IQR). All model assumptions were confirmed prior to performing all analysis.

Assessing glycaemic parameters

Fasting, total AUC_{0-240min}, incremental AUC_{0-240min} and fructosamine glycaemic response were calculated for both sitagliptin and gliclazide. The mean change in glycaemic response from baseline was also calculated.

Assessment of weight and physical activity within participants

We compared body weight between on-treatment visits using a paired T-test. A comparison between physical activity (LPA and MVPA) on sitagliptin and gliclazide therapy was calculated using Wilcoxon's Matched Pairs Rank-Sign Test as the results were non-normally distributed.

Assessing whether physical activity varies within an individual

Spearman's correlation was used to assess whether weight and physical activity (LPA and MVPA) varies within an individual between treatment visits, and between sitagliptin and gliclazide therapy.

Assessing whether clinical features are associated with physical activity

Spearman's correlation was used to assess whether clinical features such as age, BMI, weight, eGFR, diabetes duration and gender were associated with mean physical activity (LPA and MVPA) on treatment. The association of physical activity with categorical variables, including gender was assessed using Mann-Whitney U.

Assessing continuous relationship between lifestyle factors and therapeutic response to sitagliptin and gliclazide

We assessed the continuous relationship between lifestyle factors (change in weight from baseline and physical activity) and on therapy glycaemic response using linear regression. Linear regression models were adjusted for the baseline glucose measurements and drug order.

We also used univariate linear regression to assess whether differences in participant mean body weight and physical activity between on-treatment visits (sitagliptin – gliclazide) were associated with the differences in glycaemic outcomes (on therapy fasting glucose, total AUC_{0-240min} incremental AUC_{0-240min}) between study therapies, with adjustment for drug order.

5.3.1 Results - Weight

Participant weight across the study

Mean(SD) weight on sitagliptin was 89.5(15.6)kg and was 90.1(15.8)kg on gliclazide (Wilcoxon's $p < 0.0001$). The difference in participant weight between sitagliptin and gliclazide therapy was -0.63(1.5)kg. The average change in weight from baseline was -0.9(1.9)kg and -0.28(1.8)kg on sitagliptin and gliclazide respectively ($p < 0.001$), with the average total weight change across the study being -0.92(2.17). Changes in weight on therapy are shown in *Figure 11* below.

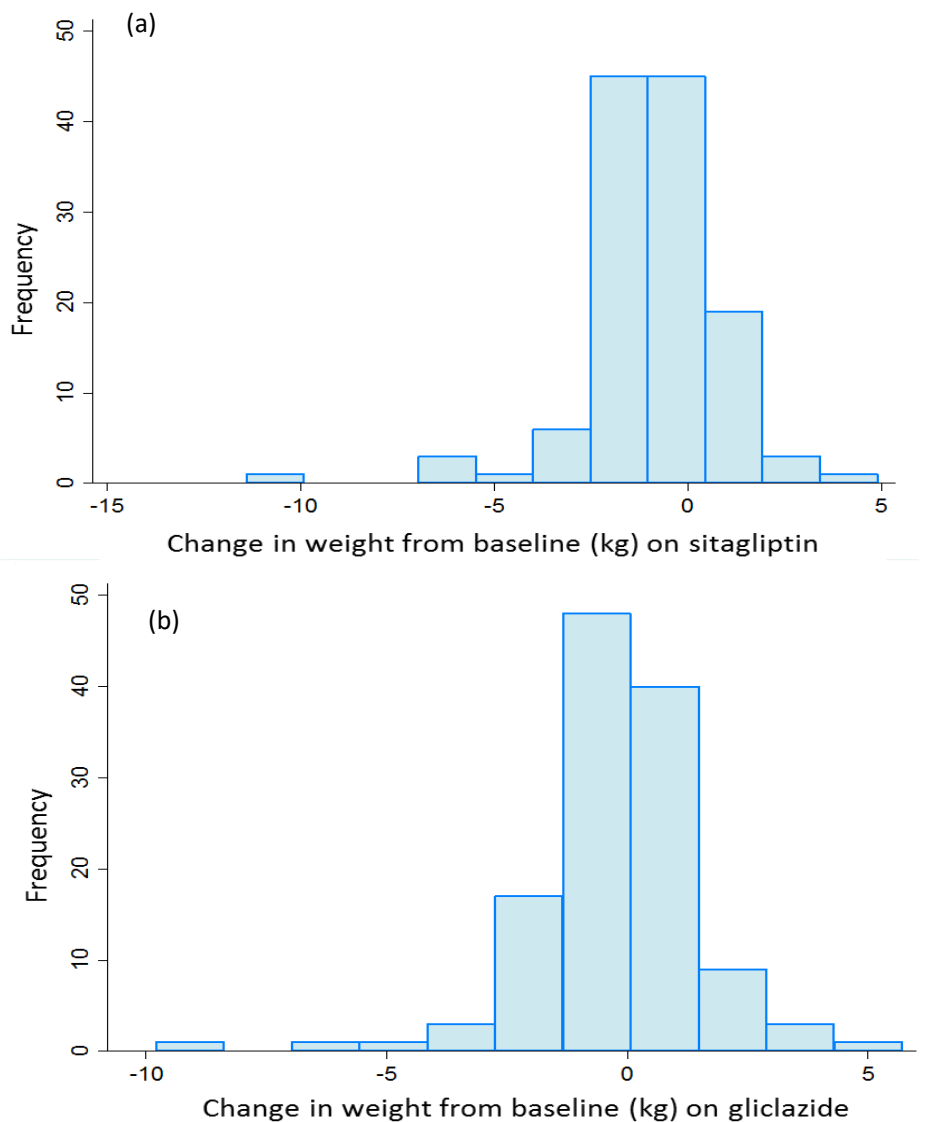


Figure 11 histograms representing the distribution of the change in weight from baseline on sitagliptin therapy (a) and gliclazide therapy (b)

Body weight is stable within an individual

As shown in *Figure 12*, body weight is stable within an individual between gliclazide and sitagliptin therapy $r=1$, $p<0.0001$.

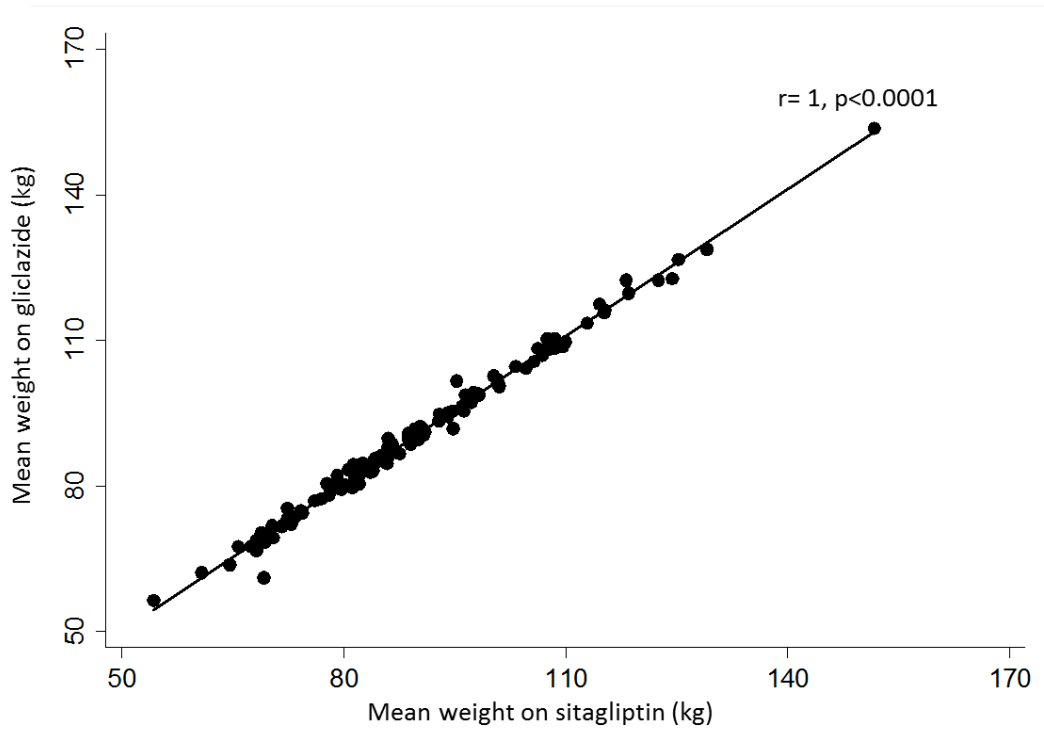


Figure 12 scatter graph showing correlation between mean weight on sitagliptin (x axis) and mean weight on gliclazide (y axis)

Differences in weight on sitagliptin and gliclazide therapy were not associated with glucose-lowering response to treatment

We found no evidence of a continuous association between change in weight from baseline on sitagliptin and gliclazide and (baseline and treatment order adjusted) fasting glucose response (*Figure 13a and b*): $\beta = 0.06$ mmol/L/min, 95%CI (-0.1,0.21) $p=0.46$ and $\beta=0.11$ mmol/L/min, 95%CI (-0.27,0.05) $p=0.17$ respectively.

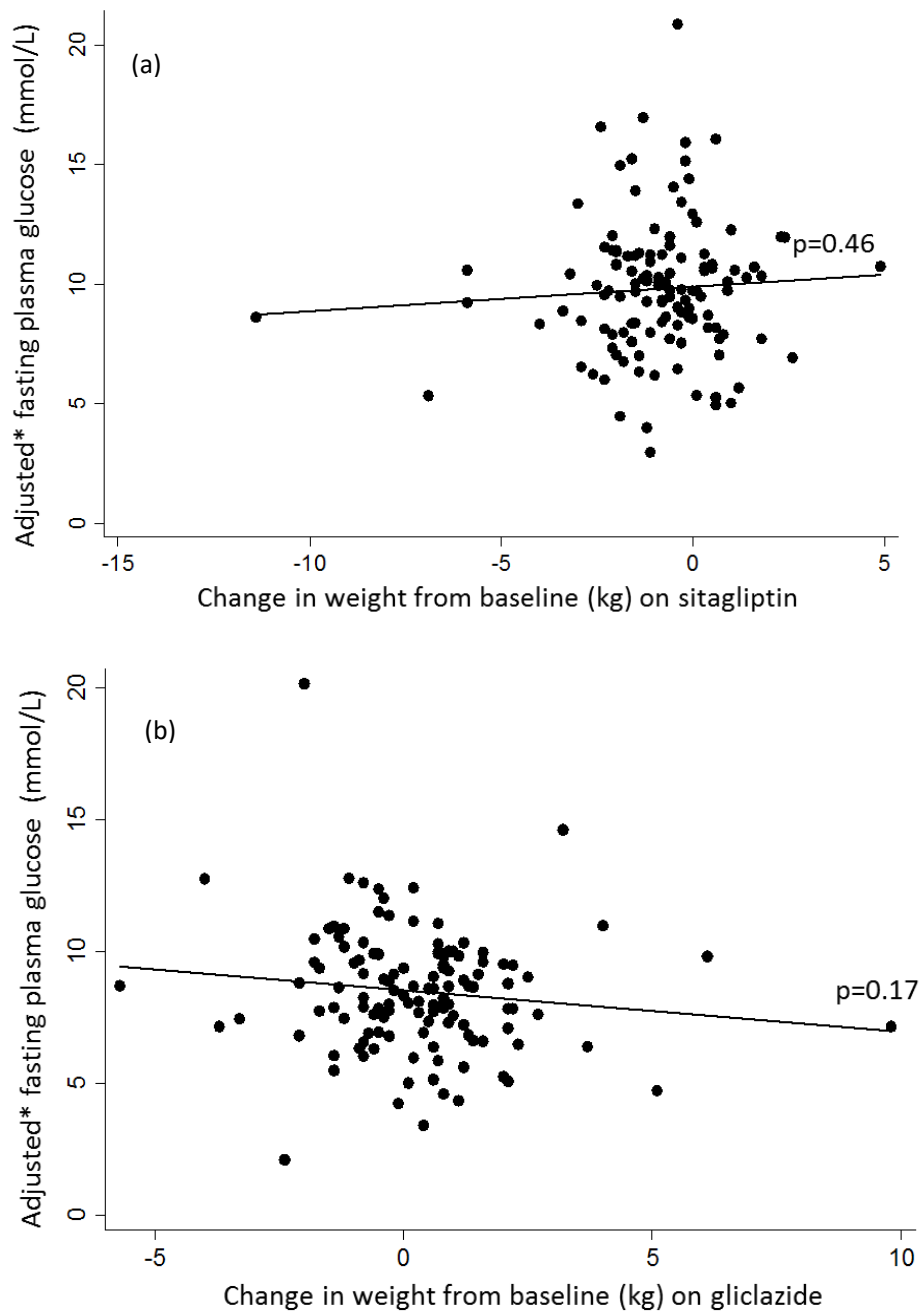


Figure 13- scatter plots showing the association between: fasting glucose levels and change in weight from baseline on sitagliptin (a) and gliclazide (b) *all glucose outcomes have been adjusted to mean baseline glucose of 9.3mmol/L for fasting glucose outcomes and were also adjusted for drug order.

As seen in *Figures 14 a* and *b* we also found no evidence of a relationship between change in weight from baseline and incremental AUC_{0-240min} glucose using the same linear regression analysis model $\beta = 11.1$, 95%CI (-18.7,40.8) $p=0.46$ for sitagliptin and $\beta = -2.32$, 95%CI (-24.6,29.3) $p=0.87$ for gliclazide. Results were similar when assessing the association between change in participant weight from baseline and total AUC_{0-240min} glucose (*Table 8*).

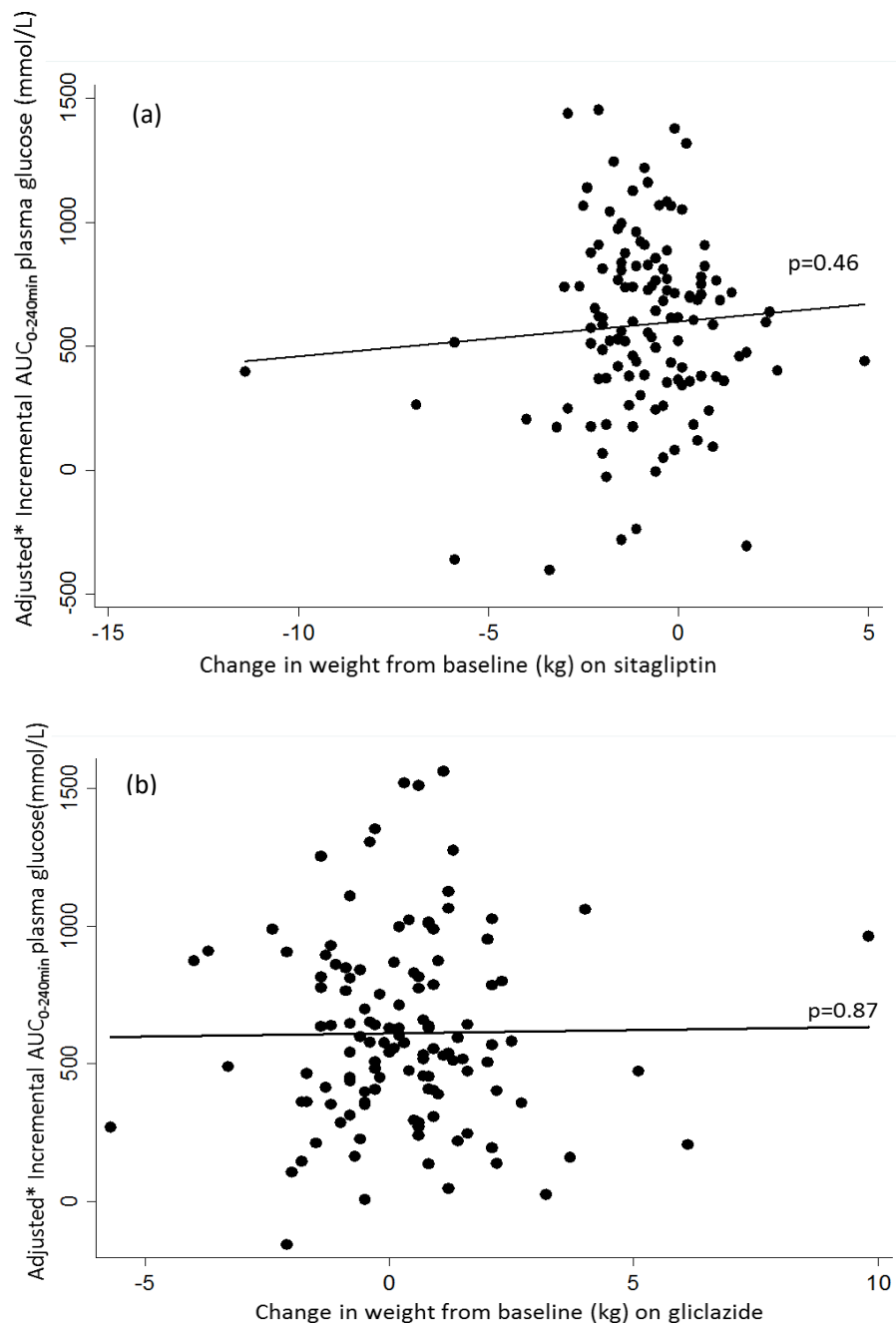


Figure 14 scatter plots showing the association between: incremental AUC_{0-240min} glucose levels and change in weight from baseline on sitagliptin (a) and gliclazide (b) *all glucose outcomes have been adjusted to mean baseline glucose of 414.7mmol/L for incremental AUC_{0-240min} outcomes and were also adjusted for drug order.

	Sitagliptin					Gliclazide				
	(n)	β	SE	(95%CI)	p value	(n)	β	SE	(95% CI)	p value
Fasting glucose	124	-0.06	0.08	-0.21, 0.1	0.46	124	-0.11	0.08	-0.27, 0.05	0.17
Total AUC _{0-240min} glucose	124	-29	24.6	-77.7, 19.8	0.24	124	-30.6	20.3	-70.9, 9.6	0.14
Incremental AUC _{0-240min} glucose	124	-11.1	15.0	-40.8, 18.7	0.46	124	2.3	13.6	-24.6, 29.3	0.87
Fructosamine	124	-1.3	2.5	-6.3, 3.6	0.6	124	-2.01	2.2	-6.4, 2.35	0.36

Table 8- Association between change in weight from baseline on sitagliptin and gliclazide and therapeutic response (fasting, total AUC0-240min, incremental AUC0-240min, and fructosamine response. All glycaemic outcomes have been adjusted for baseline glucose and drug order.

Differences in on-treatment weight between therapies do not substantially explain the differences in glucose-lowering response between study treatments.

Differential analysis that assessed whether differences in weight between sitagliptin and gliclazide therapy were associated with differences in glycaemic response between treatments (*Table 9*), revealed no evidence of a continuous association. Results from linear regression analysis were $\beta = -0.06$, 95%CI (-0.3, 0.19) $p=0.67$ for fasting response, $\beta = 29.1$, 95%CI (-5.39, 63.5) $p=0.1$ for incremental AUC_{0-240min} glucose and $\beta = -10.04$, 95%CI (-55.2, 75.3) $p=0.76$ for total AUC_{0-240min} glucose.

Sitagliptin-Gliclazide differential glucose response	(n)	β	SE	(95%CI)	p value
Fasting glucose	124	-0.06	0.13	-0.3, 0.19	0.67
Incremental AUC _{0-240min} glucose	124	29.1	17.4	-5.39, 63.5	0.1
Total AUC _{0-240min} glucose	124	10.04	33	-55.2, 75.3	0.76

Table 9- relationship between differences in weight on sitagliptin and gliclazide and the differences in glycaemic outcomes on sitagliptin and gliclazide. All linear regression outcomes have been adjusted for drug order.

5.3.2 Results - *Physical Activity*

Baseline participant characteristics

Of the 127 participants that completed both arms of the study, 89 participants provided accelerometer data for both treatment periods. Baseline characteristics of participants are listed in *Table 10*.

Baseline Characteristics	Median(IQR) (n=89)
Age (years)	68(65-74)
Ethnicity % white (n)	97.8 (87)
Gender, % male (n)	71.9(64)
Diabetes duration (years)	10(7-14)
Weight (kg)	89.5 (81.7-98)
BMI (kg/m ²)	30.7 (28.5-34.1)
eGFR (mL/min/1.73 m ²)	73 (60-85)
Baseline Fasting Glucose (mmol/L)	9.4 (8.4-11.4)
Baseline Incremental AUC 120 * Glucose (mmol/L)	423 (351-500)
Baseline Fructosamine (umol/L)	350(313-393)
Proportion of participants treated with Metformin (%)	83(93.2)

Table 10 - baseline characteristics of participants with physical activity measurements. Results expressed as median (IQR) unless specified otherwise.

Physical activity was stable between treatments

The average physical activity did not differ between participants receiving sitagliptin and gliclazide. Median (IQR) of LPA on sitagliptin and gliclazide was 159(117.3-219.3)minutes/day and 164.4(131.4-209.7)minutes/day respectively (p for comparison=0.49). Average MVPA was 20.1(10.9-40.1)minutes/day and 22.3(13.2-44.1)minutes/day (p=0.48) for sitagliptin and gliclazide.

Physical activity does not vary substantially within an individual between treatment visits or according to study therapy

When assessing whether physical activity varied within an individual according to the study therapy, a significant correlation was noted between light physical activity on sitagliptin and gliclazide, $r = 0.8$ ($p < 0.0001$) and between moderate activity on sitagliptin and gliclazide therapy $r = 0.79$ ($p < 0.0001$) (Figures 15 a and b respectively).

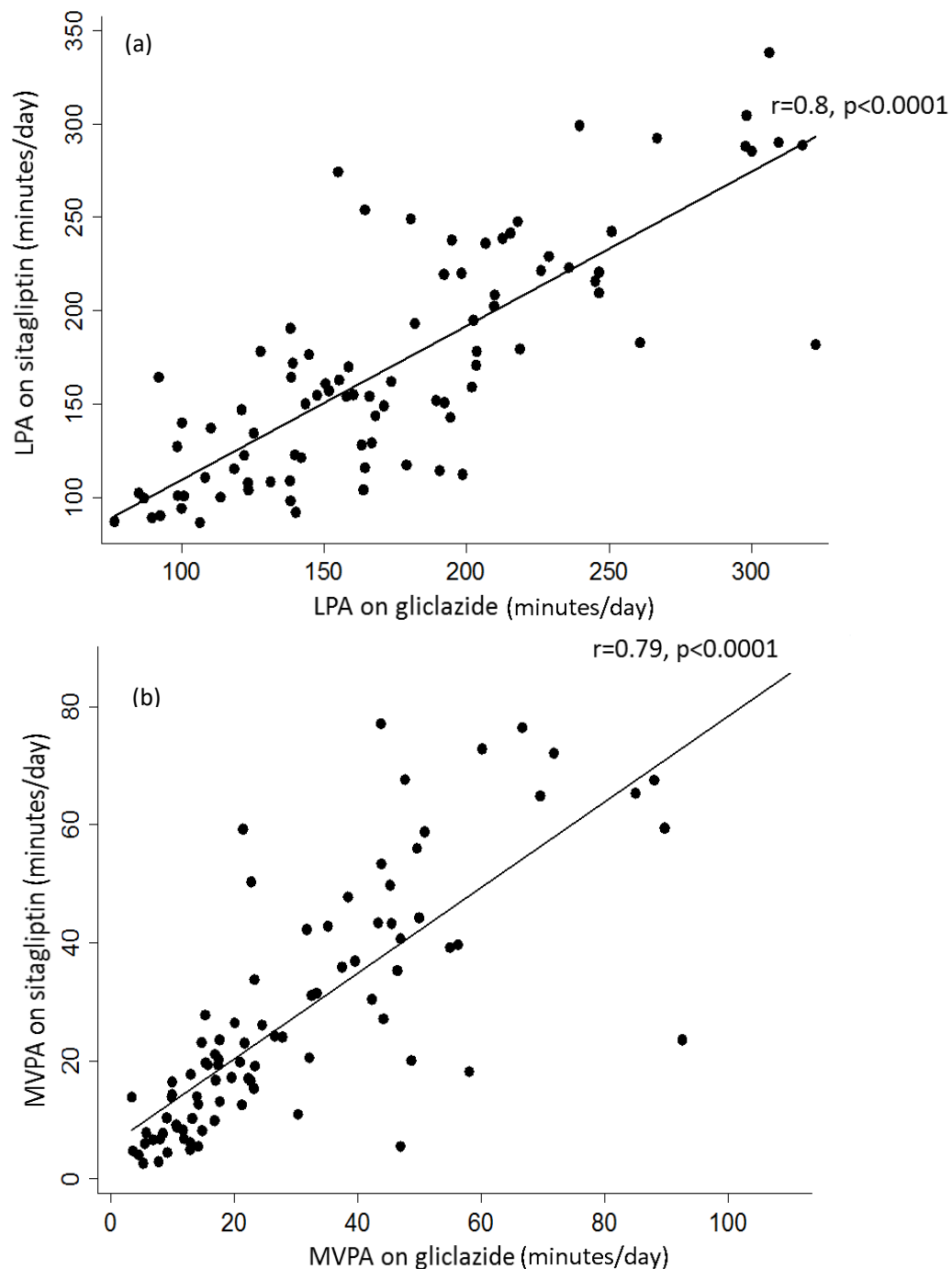


Figure 15- scatter graphs showing the association between light (a) and moderate-to-vigorous (b) physical activity during treatment period 1 and treatment period 2

Increased age and worsening renal function is associated with reduced physical activity.

The association between baseline characteristics and physical activity are shown in *Table 11*. Our results show that age and eGFR both strongly correlated with physical activity (LPA and MVPA).

Our results show that as age increases, frequency of physical activity decreases: $r(p\text{-value})$ was $-0.22(0.03)$ and $-0.37(0.0004)$ for LPA and MVPA respectively. With regards to renal function, our results show that an increased eGFR, strongly correlates with an increase in physical activity, $r(p\text{-value})$ $0.35(0.0007)$ and $0.33(0.0014)$. Other clinical determinants including gender, BMI, weight and duration of diabetes were not associated with physical activity (*Table 11*).

Clinical Characteristics	Light physical activity Spearman's r (p-value)	Moderate-to-vigorous activity Spearman's r (p-value)
Age (years)	$-0.22(0.03)$	$-0.37(0.0004)$
Diabetes duration (years)	$-0.005(0.96)$	$-0.14(0.19)$
Weight (kg)	$-0.15(0.17)$	$0.03(0.75)$
BMI (kg/m^2)	$-0.08(0.49)$	$0.04(0.73)$
eGFR ($\text{mL}/\text{min}/1.73 \text{ m}^2$)	$0.35(0.0007)$	$0.33(0.0014)$
Gender	0.87	0.024

Table 11-relationship between baseline clinical characteristics and physical activity (LPA and MVPA) using Spearman's correlation, with the exception for gender (a categorical variable) which was assessed using Mann-Whitney U.

Light physical activity is not associated with therapeutic response to sitagliptin or gliclazide therapy

As shown in *Figures 16a and 16b*, we found no evidence of a continuous relationship between LPA and adjusted fasting glucose response to sitagliptin $\beta = -0.0009$ mmol/L, 95%CI (-0.005, 0.005) $p = 0.97$ or to gliclazide $\beta = -0.002$ mmol/L, 95%CI (-0.008, 0.003) $p = 0.38$. We also found no evidence of a continuous association between LPA and post-meal glycaemic response to sitagliptin or gliclazide: $\beta = 0.09$ mmol/L/min, 95%CI (-0.31, 0.51) $p = 0.65$ and $\beta = 0.17$ mmol/L/min, 95%CI (-0.16, 0.51) $p = 0.3$ respectively (*Figure 16c and 16d*) with similar results assessing total AUC_{0-240min} and fructosamine response (*Table 12*).

Moderate-to-vigorous physical activity is not associated with therapeutic response to sitagliptin or gliclazide

Univariate linear regression analysis revealed no evidence of a continuous association between MVPA and (baseline and drug order) adjusted fasting glucose for sitagliptin $\beta = -0.004$ mmol/L, 95%CI(0.02, 0.01) $p = 0.59$ or for gliclazide $\beta = -0.12$ mmol/L, 95%CI (-0.03, 0.03) $p = 0.11$ (*Figure 17a and b*). Similarly, as shown in *Figures 17c and d* our results also show no evidence of an association between MVPA and incremental AUC_{0-240min} glucose response to sitagliptin $\beta = 0.71$ mmol/L/min, 95%CI (-2.06, 3.47) $p = 0.61$ or to gliclazide $\beta = 0.29$ mmol/L/min, 95%CI (-2.02, 2.59) $p = 0.81$. There was also no evidence of a relationship between MVPA and total AUC_{0-240min} glucose and fructosamine response (*Table 13*).

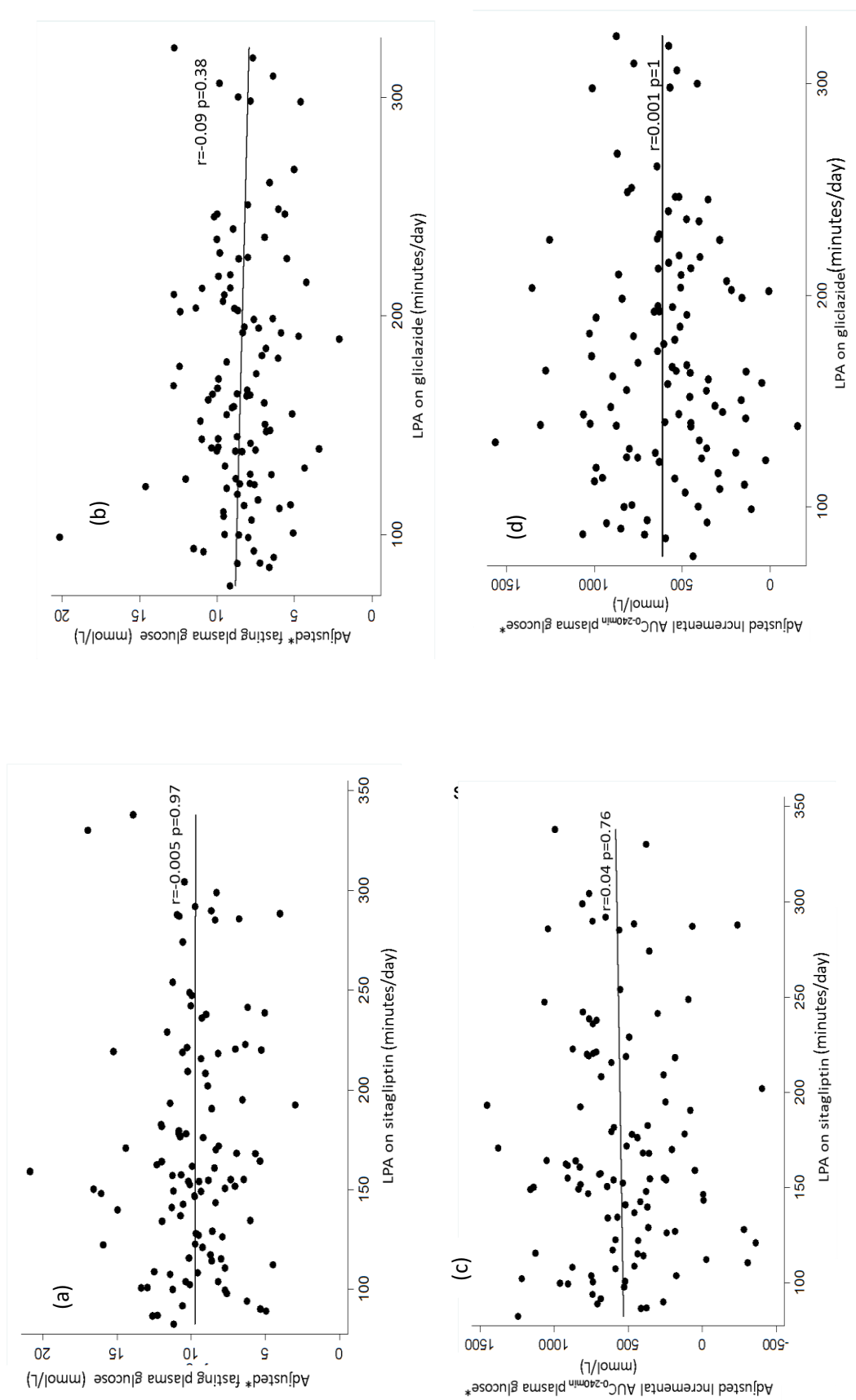


Figure 16 : scatter plots showing the association between: light physical activity and fasting plasma glucose for sitagliptin (a) and gliclazide (b) and light physical activity and incremental AUC_{0-240min} for sitagliptin (c) and gliclazide (d). *all glucose outcomes have been adjusted to mean baseline glucose of 9.3mmol/L for fasting glucose outcomes and 417.4 for incremental AUC_{0-240min}. All glucose outcomes were also adjusted for drug order.

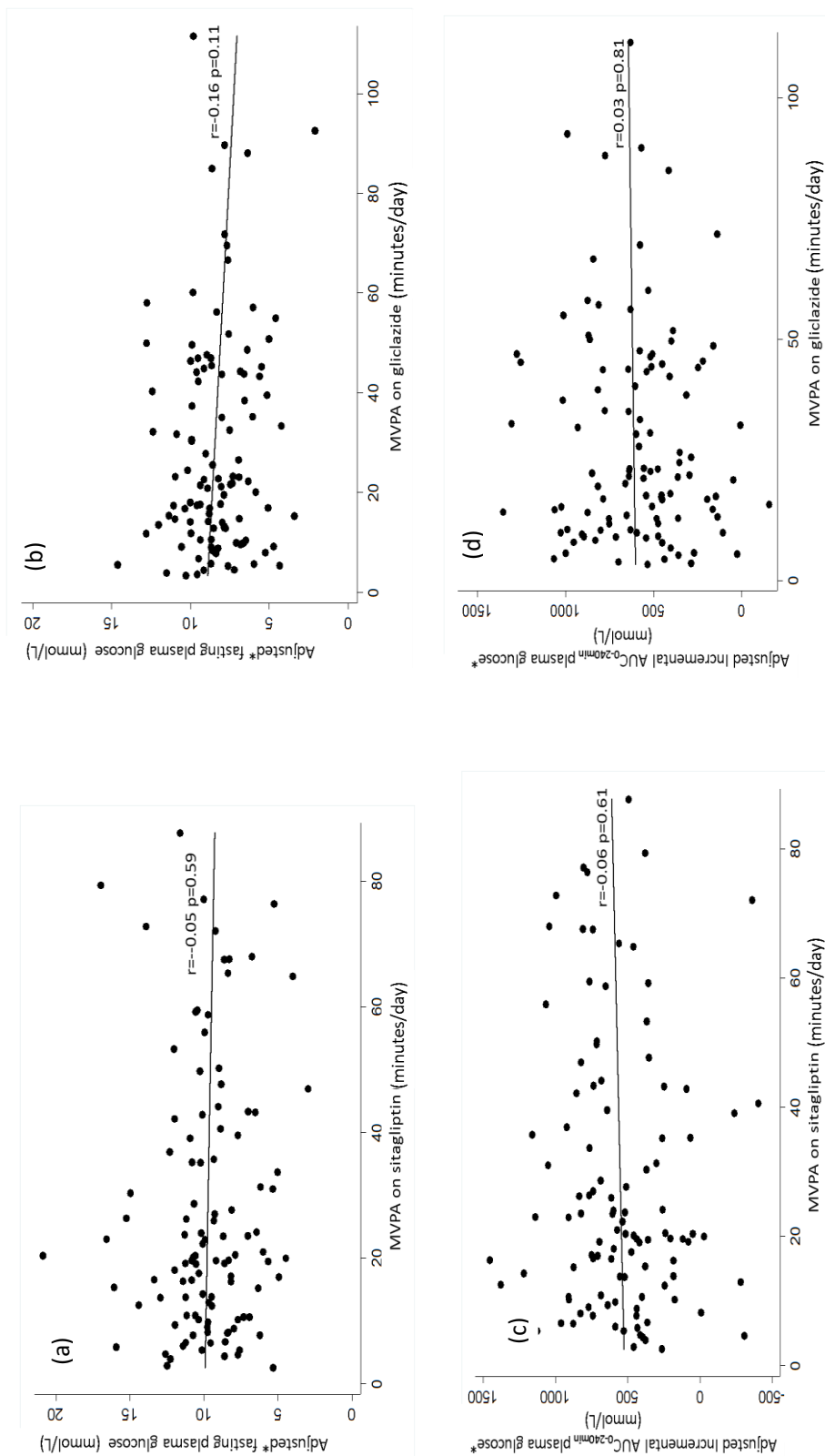


Figure 17- scatter plots showing the association between: moderate-to-vigorous physical activity and fasting plasma glucose for sitagliptin (a) and gliclazide (b) and moderate-to-vigorous physical activity and incremental $AUC_{0-240min}$ for sitagliptin (c) and gliclazide (d). **all glucose outcomes have been adjusted to mean baseline glucose of 9.3mmol/L for fasting glucose outcomes and 417.4 for incremental $AUC_{0-240min}$. All glucose outcomes were also adjusted for drug order.*

Outcome (Sitagliptin)	(n)	β	SE	t	(95% CI)	p value
Fasting glucose	89	-0.0009	0.002	-0.03	-0.005, 0.005	0.97
Total AUC _{0-240min} glucose	89	0.04	0.79	0.06	-1.52, 1.6	0.96
Incremental AUC _{0-240min} glucose	89	0.15	0.47	0.23	-0.78, 1.08	0.76
Fructosamine	89	0.12	0.09	1.32	-0.06, 0.29	0.19
Outcome (Gliclazide)	(n)	β	SE	t	(95% CI)	p value
Fasting glucose	89	-0.002	0.003	-0.89	-0.008, 0.003	0.38
Total AUC _{0-240min} glucose	89	-0.6	0.68	-0.88	-1.94, 0.74	0.38
Incremental AUC _{0-240min} glucose	89	-0.002	0.44	-0.16	-0.88, 0.87	1
Fructosamine	89	-0.04	0.08	-0.49	-0.2, 0.122	0.62

Table 12- continuous association between MVPA and glycaemic response to sitagliptin (above) and gliclazide (below). Analysis performed using linear regression with outcomes adjusted for baseline glucose and drug order.

Outcome (Sitagliptin)	(n)	β	SE	t	(95% CI)	p value
Fasting glucose	89	-0.004	0.008	-0.54	-0.02, 0.01	0.59
Total AUC _{0-240min} glucose	89	-0.26	2.34	-0.12	-4.9, 4.4	0.91
Incremental AUC _{0-240min} glucose	89	0.71	1.4	0.55	-2.06, 3.47	0.61
Fructosamine	89	0.19	0.26	0.72	-0.33, 0.71	0.47
Outcome (Gliclazide)	(n)	β	SE	t	(95% CI)	p value
Fasting glucose	89	-0.12	0.01	-1.59	-0.03, 0.03	0.11
Total AUC _{0-240min} glucose	89	-2.4	1.81	-1.33	-5.98, 1.18	0.19
Incremental AUC _{0-240min} glucose	89	0.29	1.16	0.26	-2.02, 2.59	0.81
Fructosamine	89	-0.26	0.21	-1.22	-0.69, 0.16	0.23

Table 13- continuous association between LPA and glycaemic response to sitagliptin (above) and gliclazide (below). Analysis performed using linear regression with outcomes adjusted for baseline glucose and drug order.

Differences in physical activity between sitagliptin and gliclazide therapy do not substantially explain the differences in glycaemic response between treatment regimens

The differences in the frequency of light physical activity between treatments were not associated with the differences in observed glycaemia between sitagliptin and gliclazide, linear regression $\beta = 0.002$, 95%CI (-0.01, 0.01) $p = 0.74$ and $\beta = 0.93$, 95%CI (-0.69, 2.55) $p = 0.26$ for fasting glucose and incremental $AUC_{0-240min}$ glucose respectively (*Table 14*).

Similarly we also found no evidence of a continuous association between differences in MVPA between sitagliptin and gliclazide therapy and differences in fasting or incremental $AUC_{0-240min}$ glucose response: $\beta = -0.002$, 95%CI (-0.03, 0.03) $p = 0.89$, and $\beta = 2.7$, 95%CI (-1.66, 7.07) $p = 0.22$, respectively (*Table 15*).

Light physical activity Sitagliptin-Gliclazide differential glucose response		(n)	β	SE	(95% CI)	p value
Fasting glucose		89	0.002	0.005	-0.01, 0.01	0.74
Total AUC _{0-240min} glucose		89	1.56	1.45	-1.3, 4.45	0.29
Incremental AUC _{0-240min} glucose		89	0.93	0.82	-0.69, 2.55	0.26
Moderate-to-vigorous physical activity Sitagliptin-Gliclazide differential glucose response		(n)	β	SE	(95% CI)	p value
Fasting glucose		89	-0.002	0.014	-0.03, 0.03	0.89
Total AUC _{0-240min} glucose		89	2.22	4	-5.7, 10.2	0.58
Incremental AUC _{0-240min} glucose		89	2.7	2.19	-1.66, 7.07	0.22

Table 14- results from linear regression analysis, showing no significant association between differences in glycaemic response between sitagliptin and gliclazide therapy and differences between light physical activity (top) and moderate-to-vigorous physical activity (bottom) between therapies. Glycaemic outcomes were adjusted for drug order.

5.4 Discussion

Our results have shown that measurable differences in weight and physical activity between sitagliptin and gliclazide therapy do not substantially explain the differences in short term glucose-lowering response between these treatments therapy. We have shown that weight and physical activity is stable within individual and show no continuous associated with therapeutic response to sitagliptin and gliclazide.

We have identified that reduced physical activity is associated with increased age and reduced renal function, which is consistent with current evidence within the literature [1–3].

Comparison of our findings with weight studies

Our weight findings are consistent with studies which show that variation in weight is not associated with response to GLP-1 therapy [4], but inconsistent with studies that show weight change (achieved by dietary interventions) is associated with improvement in glycaemic control [5, 6].

Few studies have investigated the direct relationship between physical activity and therapeutic response to diabetes medications. Most of the studies that do investigate the effects of intensive exercise interventions in controlled settings. These studies show that physical activity leads to a lower glucose after exercise when combined with diabetes therapy, compared to exercise alone or oral therapy, however their findings are limited by their small sample size.

One small study conducted on 8 patients with T2D investigated whether there were cumulative glucose-lowering effects of a 60 minutes of an acute submaximal exercise intervention (performed by using a bicycle ergometer) to 7mg glibenclamide compared with glibenclamide alone [7]. Its results showed that reductions in plasma glucose were significantly higher on days where participants combined exercise and glibenclamide compared exercise alone ($p < 0.05$) [7].

A larger, randomised control trial performed on 167 patients with T2D also investigated the metabolic effects of exercise on glycaemic response to sulphonylurea therapy [8]. In this study, participants were randomised in a double-blinded manner to either glimepiride 3mg OD or glibenclamide 10mg OD

for a period of 14-28 days and then further openly randomised to groups with or without an exercise intervention. The exercise intervention consisted of riding a bicycle ergometer at a pulse rate of 120 beats per minute, for a period of 1 hour. Paired comparisons revealed a statistically significant reduction in post-meal AUC plasma glucose levels in participants in the exercise and took SU therapy compared to participants who only took SU therapy [8].

Findings from the Early Activity in Diabetes (Early ACTID) study provide a more robust assessment of daily physical activity on glycaemic response in the T2D cohort. The Early ACTID Study is a large, randomised controlled trial that investigated the effects of intensive physical activity advice and diet advice on glycaemic control in 593 individuals, newly diagnosed with T2D [5]. Participants were randomised to one of 3 intervention arms including: usual care (n=99), intensive diet intervention (n=248) and an intensive diet + physical activity intervention (n=246). Approximately 40% of participants were on at least one glucose lowering agent. The diet intervention involved structured dietary consultations every 3 months which aimed to encourage 5-10% weight loss, whilst the physical activity intervention involved requesting participants to perform at least 30 minutes of brisk walking 5 days per week [5]. Physical activity was calculated using an accelerometer and pedometer readings. Its results showed that although the dietary and dietary + physical activity interventions significantly improved HbA1c response compared to the control group ($p=0.05$ and $p<0.001$ respectively), the addition of physical activity showed no additional benefit in improving glycaemia [5].

These findings are supported by those from the Italian Diabetes and Exercise Study (IDES). IDES is a randomised controlled trial which investigated the effects of structured physical activity advice and a supervised intensive exercise intervention on HbA1c response in 606 patients with T2D [9] (with approximately 80% of participants were taking oral hypoglycaemic therapy). Participants randomised to the supervised exercise intervention arm (150 minutes of progressive mixed (aerobic and resistance) training per week) performed more physical activity had a significant reduction in HbA1c response ($p<0.001$) compared to participants who received physical activity counselling alone [9].

Although the two latter studies [5, 9] have not directly assessed the effects of physical activity on the variation in therapeutic response to hypoglycaemic agents, they demonstrate that daily physical activity did not substantially influence glycaemia in the T2D cohort.

Strengths and limitations

Our analysis is strengthened by the interventional study design from which the data was collated. Furthermore our weight and accelerometer data reflects daily lifestyle behaviours typical of the T2D cohort, with robust measurements of physical activity taken over multiple time points.

The short duration of the MASTERMIND study limits our findings. Although our results demonstrate that weight and physical activity within participants remained stable across the study, it is unclear whether longer intervention periods would have captured more variation in these lifestyle factors and thus their effects on response to sitagliptin and gliclazide therapy. Furthermore, the 4-week intervention period precludes the assessment of HbA1c response (the gold standard measurement for monitoring glycaemic control) thus challenging the implications of our findings to clinical practice.

Our findings on body weight and therapeutic response are limited by not including analysis on body composition; thus, the extent to which our findings reflect true body weight (including body fat and muscle) as opposed to variations in “noise” (including hydration status, daily physical activity patterns and dietary intake) is uncertain. Measuring participants’ body weight in a fasted state however, is likely to have controlled this “noise”.

Our findings on physical activity and therapeutic response is limited by not including sedentary time and sleep time in our analysis. Due to the way physical activity was analysed, it was not possible to distinguish between these two variables. Evidence within the literature suggests that increased quality sleep is associated with improved health outcomes in diabetes[10]. Furthermore, emerging data indicates that interrupting sedentary time with standing or light physical activity may improve glycaemia and insulin sensitivity in patients T2D. Investigating the effects of sleep and sedentary time on response to diabetes medication may therefore provide avenue for future research [11].

5.5 Conclusion

In conclusion, our findings demonstrate that lifestyle factors (weight and physical activity) are relatively stable within individuals with T2D. Evidence from our analysis has shown that short-term differences in weight and physical activity between and within an individual do not substantially explain the variation in short term glycaemic response to sitagliptin and gliclazide therapy. The implications of these findings suggest that measuring weight and physical activity in control trials for stratified diabetes is unlikely to be helpful.

5.6 References

1. Stump CS (2011) Physical Activity in the Prevention of Chronic Kidney Disease. *Cardiorenal Med* 1:164–173 . doi: 10.1159/000329929
2. Tsai Y-C, Chen H-M, Hsiao S-M, et al (2017) Association of physical activity with cardiovascular and renal outcomes and quality of life in chronic kidney disease. *PLOS ONE* 12:e0183642 . doi: 10.1371/journal.pone.0183642
3. Slee AD (2012) Exploring metabolic dysfunction in chronic kidney disease. *Nutr Metab (Lond)* 9:36 . doi: 10.1186/1743-7075-9-36
4. Prasad-Reddy L, Isaacs D (2015) A clinical review of GLP-1 receptor agonists: efficacy and safety in diabetes and beyond. *Drugs Context* 4: . doi: 10.7573/dic.212283
5. Andrews RC, Cooper AR, Montgomery AA, et al (2011) Diet or diet plus physical activity versus usual care in patients with newly diagnosed type 2 diabetes: the Early ACTID randomised controlled trial. *Lancet* 378:129–139 . doi: 10.1016/S0140-6736(11)60442-X
6. Phung OJ, Scholle JM, Talwar M, Coleman CI (2010) Effect of noninsulin antidiabetic drugs added to metformin therapy on glycemic control, weight gain, and hypoglycemia in type 2 diabetes. *JAMA* 303:1410–1418 . doi: 10.1001/jama.2010.405
7. Larsen JJ, Dela F, Madsbad S, et al (1999) Interaction of sulfonylureas and exercise on glucose homeostasis in type 2 diabetic patients. *Diabetes Care* 22:1647–1654
8. Massi-Benedetti M, Herz M, Pfeiffer C (1996) The Effects of Acute Exercise on Metabolic Control in Type II Diabetic Patients Treated with Glimepiride or Glibenclamide. *Hormone and Metabolic Research* 28:451–455 . doi: 10.1055/s-2007-979836
9. Balducci S, Zanuso S, Nicolucci A, et al (2010) Effect of an intensive exercise intervention strategy on modifiable cardiovascular risk factors in subjects with type 2 diabetes mellitus: a randomized controlled trial: the

Italian Diabetes and Exercise Study (IDES). *Arch Intern Med* 170:1794–1803 . doi: 10.1001/archinternmed.2010.380

10. Arora T, Taheri S (2015) Sleep Optimization and Diabetes Control: A Review of the Literature. *Diabetes Ther* 6:425–468 . doi: 10.1007/s13300-015-0141-z
11. Duvivier BMFM, Schaper NC, Hesselink MKC, et al (2017) Breaking sitting with light activities vs structured exercise: a randomised crossover study demonstrating benefits for glycaemic control and insulin sensitivity in type 2 diabetes. *Diabetologia* 60:490–498 . doi: 10.1007/s00125-016-4161-7

Chapter 6

Chapter 6: is response to glucose-lowering therapy specific to an individual?

6.1 Introduction

There is huge variation in response to glucose-lowering therapy in T2D, with some individuals showing a marked response to one drug but with others eliciting a poor response to the same drug. The extent to which non-response reflects an intrinsic lack of response (attributed to reproducible biological factors which may be predicted) as opposed to extrinsic factors (such as variations in lifestyle or medication concordance) is yet to be determined. For example, it is possible that individuals with high insulin resistance have poor response to all glucose lowering therapies, a finding that would challenge a precision medicine approach to T2D. Stratification will only be effective in T2D if it can be proven that poor response to one hypoglycaemic agent is associated with a good response to another agent with a different mechanism of action (as opposed to a lack of response to all hypoglycaemic agents).

We aimed to determine whether response to glucose-lowering therapy may have a biological basis by investigating glycaemic responses between stopping sulphonylurea therapy and starting sulphonylurea and DPP-4 inhibitor therapy. We also assessed whether response is specific to a drug, by investigating whether the glucose-lowering response to one diabetes medication (gliclazide) is associated with the same response to another agent, with a different mechanism of action (sitagliptin).

6.2 Methods

6.2.1 Study design

To assess whether response to glucose-lowering medication is a biological characteristic of an individual, we assessed the relationship between the change in fasting glycaemia 1 week after stopping sulphonylurea therapy (during the initial washout period of the MASTERMIND study) and the change fasting glucose response after starting a sulphonylurea or DPP-4 inhibitor therapy in the same individuals as part of the study protocol.

Although fasting glucose measurements were available at 2 weeks after SU withdrawal (visit 3), we assessed fasting glucose response at 1 week because the 2 week measurement represents the baseline for fasting glycaemic response for participants' first drug. Hence, using the fasting glucose measurement after the 2 week washout period would consequently introduce a false correlation between change in fasting glucose after withdrawing SU therapy and subsequent on-treatment response (as the same fasting glucose measurement would have been used to calculate response in both outcomes).

6.2.2 Statistical analysis

Is the change in glycaemia after stopping sulphonylurea therapy associated with change in glycaemia observed in starting gliclazide and sitagliptin therapy?

Spearman's correlation and linear regression were used to assess the relationship between the change in fasting glycaemia after discontinuing sulphonylurea therapy and the mean change in fasting glycaemic response on study therapy. Linear regression models were adjusted for drug order.

Assessing whether the glucose-lowering response to sitagliptin is associated with the glucose lowering response to gliclazide

The relationship between mean change in on-treatment fasting glucose response to sitagliptin and gliclazide therapy was assessed using spearman's correlation and linear regression (with adjustment for drug order).

6.3 Results

Glycaemic response after stopping sulphonylurea therapy is associated with glycaemic response to restarting sulphonylurea therapy

Figure 19 shows a significant correlation between fasting glycaemia observed 1 week after stopping sulphonylurea therapy and the reduction in fasting glycaemia upon restarting sulphonylurea therapy $r=0.19$, $p=0.03$, an association supported by linear regression analysis: $\beta= 0.22$ mmol/L, 95%CI (0.01, 0.41) $p=0.04$. This correlation indicates that an increase in fasting glycaemia from stopping SU therapy is associated with the reduction in fasting glycaemia upon re-starting SU therapy.

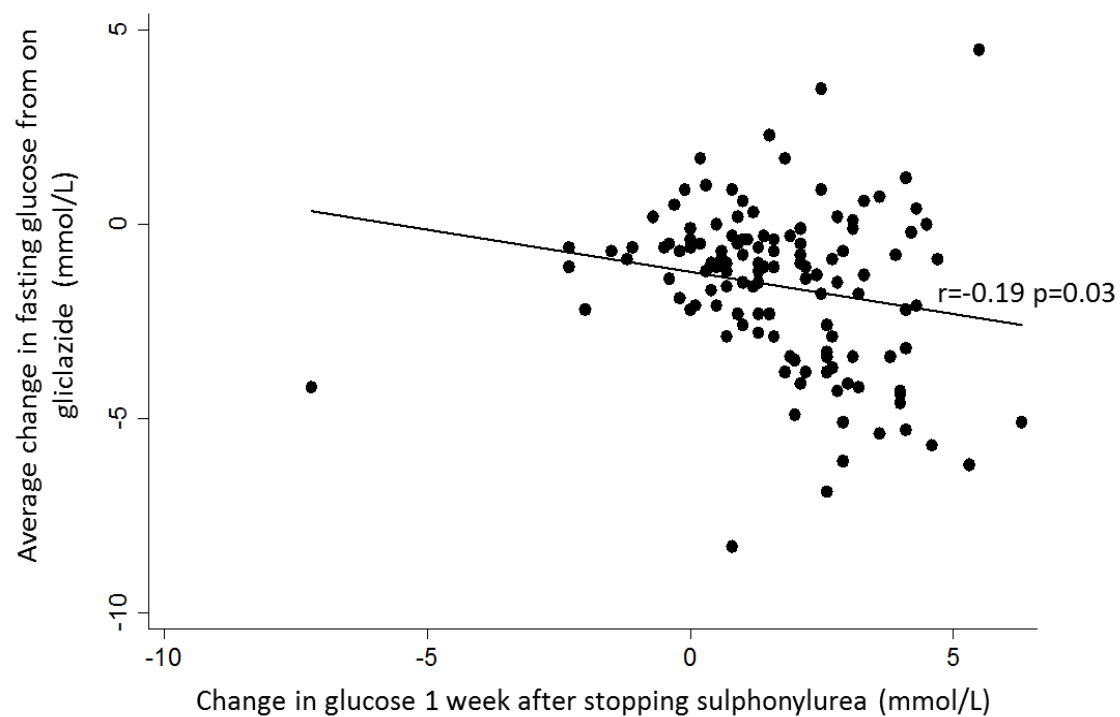


Figure 18- scatter graph showing a significant correlation within an individual between change in fasting glucose 1 week after withholding sulphonylurea therapy (x axis) and average change in fasting glucose on gliclazide therapy, administered per study protocol (y-axis).

Glycaemic response to withdrawing sulphonylurea therapy is not associated with glycaemic response to initiating sitagliptin therapy

Contrastingly, our results show no evidence of a relationship between the change in fasting glycaemia after stopping sulphonylurea and the change in fasting glycaemia to starting sitagliptin therapy: $r = -0.02$, $p = 0.79$ (Figure 20), linear regression: $\beta = -0.02 \text{ mmol/L}$ ($-0.19, 0.15$) $p = 0.78$.

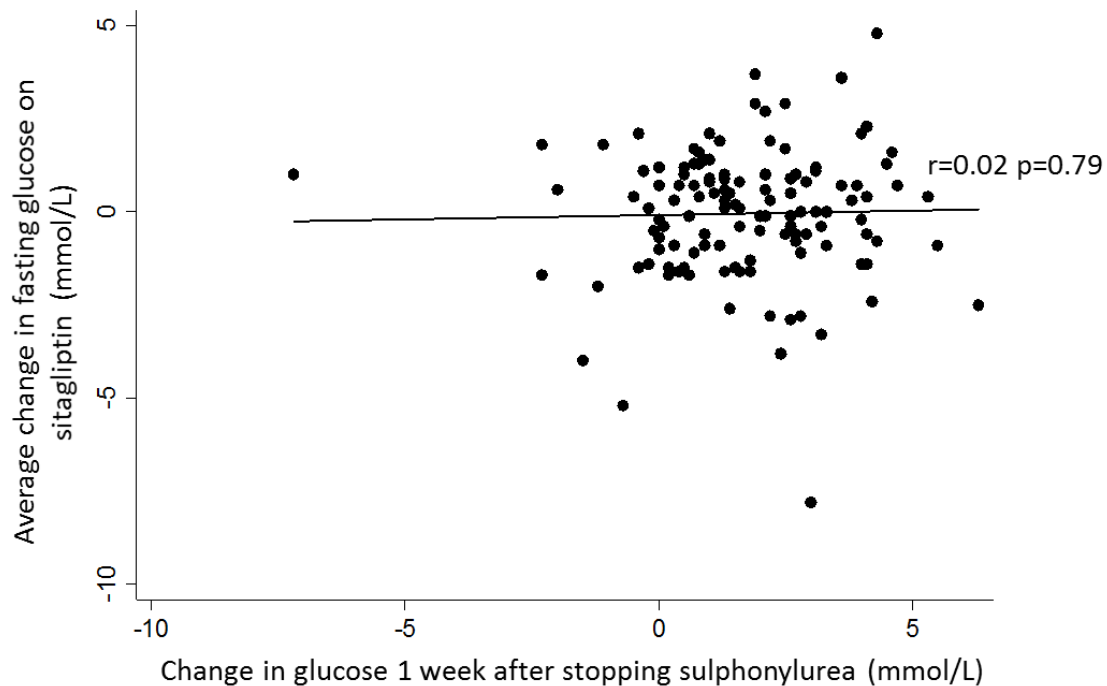


Figure 19- scatter graph showing no evidence of a relationship between change in fasting glucose 1 week after withholding sulphonylurea therapy (x axis) and average change in fasting glucose on sitagliptin therapy within an individual (y-axis).

Fasting glycaemic response to sitagliptin is not associated with glycaemic response to gliclazide

As shown in *Figure 21*, we found no evidence of a significant correlation between the fasting glycaemic responses to sitagliptin and gliclazide therapy: $r=0.008$, $p=0.99$.

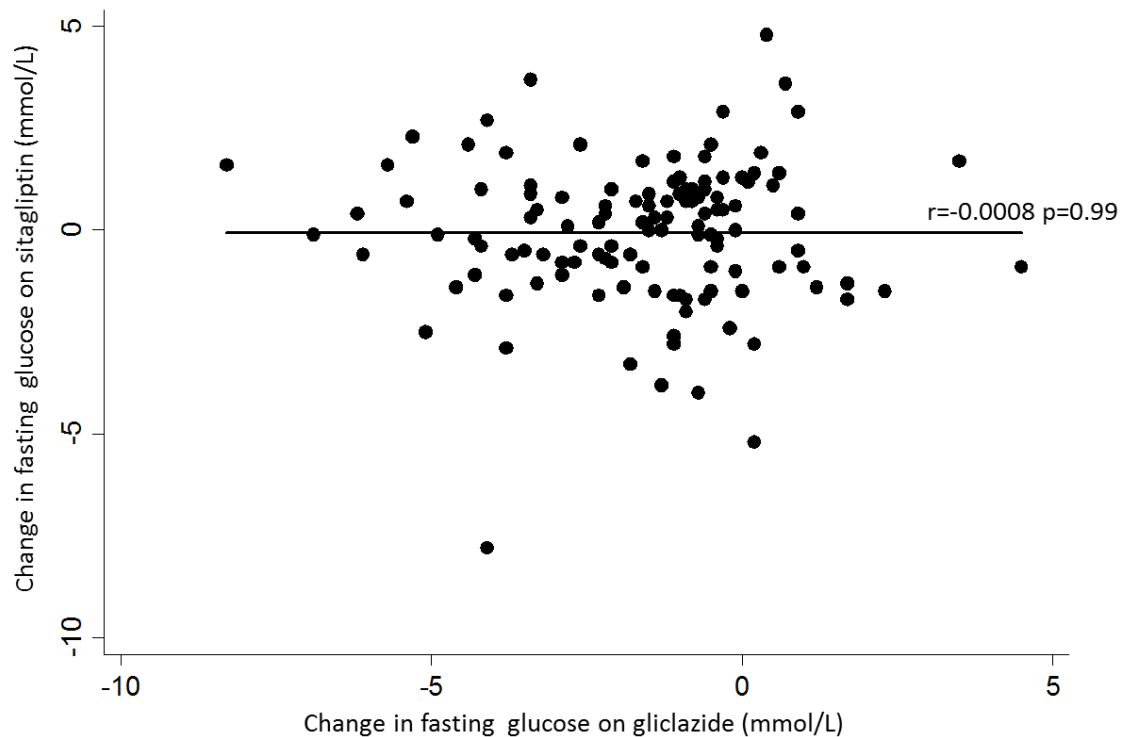


Figure 20- scatter graph showing no significant correlation between fasting glycaemic responses to gliclazide (x- axis) and sitagliptin therapy (y-axis).

6.4 Discussion

We have shown that the change in fasting glycaemia after stopping sulphonylurea therapy is a strong predictor of fasting glycaemic response when recommencing SU treatment, but is not associated with the fasting glycaemic response to sitagliptin therapy. The association between glycaemic responses to stopping and starting sulphonylurea therapies suggests that there is in part, a biological basis to the variation in therapeutic response to sulphonylurea therapy. These findings also suggest that the variation in response is not solely influenced by background noise (such as variations in diet, exercise and medication adherence). Our results have shown that an individual's glycaemic response may be specific to a drug as an individual's fasting response to sitagliptin was not associated with their observed fasting glycaemic response to gliclazide.

Comparison with other studies

Previous studies have investigated the changes in glycaemia after stopping sulphonylurea therapy [1–3], however they did not investigate whether this change is associated with the glycaemic response to restarting sulphonylurea therapy or to initiating a different glucose lowering agent within an individual. Furthermore, we have found no other interventional studies that have investigated whether response to one glucose lowering agent is associated with the glucose-lowering response to another agent, with a differing mechanism of action within an individual. This largely attributed to a lack of studies investigating inter-individual variation to glucose lowering therapy, specifically randomised control crossover trials (which allow direct comparisons of therapeutic response to different medications within an individual).

Strengths and limitations

A key strength is the novelty of our findings; to our knowledge no other study has investigated the association in glycaemic responses between stopping and starting a glucose lowering agent within an individual.

Unfortunately our findings have several limitations. Firstly, when investigating the relationship between glycaemic responses after stopping sulphonylurea therapy and starting gliclazide or sitagliptin therapy, we were limited to using fasting glycaemic measurements after one week of withholding sulphonylurea therapy (visit 2), rather than the fasting measurement after the full two week washout

period (visit 3). This is because the 2 week measurement represents the baseline for response for participants' first study therapy, which would introduce a false correlation (and therefore bias) when assessing the association between change in glycaemia on withdrawal and subsequent response (due to use of the same measurement in both response outcome). Despite this, the change in fasting glycaemia after one week of stopping sulphonylurea therapy was similar to that after two weeks (mean(SD) change in fasting glucose was 1.7(1.8)mmol/L after one week and 1.6(2.1)mmol/L after two weeks) suggesting that the majority of the glycaemic effect from SU withdrawal occurred within the first week.

Secondly, although prior to commencing the study the majority of participants were on an identical gliclazide regimen as per the study protocol (96%), some participants observed different regimens, with few participants on different sulphonylurea agents (including glipizide and tolbutamide). Thus, the effects of these different sulphonylurea therapies on fasting glucose response remains unclear.

Our findings are further limited by only having fasting glucose measurements for the start and end of each treatment period (with post-prandial glycaemic assessments only conducted at the end of each treatment period). This meant we were unable to calculate the change in post-prandial glycaemia for each treatment period. Unlike gliclazide, sitagliptin has little effect on reducing fasting glucose and mainly exerts its glucose-lowering effects on post prandial glucose levels. Unfortunately as the change in post-meal glucose response to each study therapy could not be assessed, our analysis has been unable to determine the full therapeutic effect of sitagliptin. Thus, it remains unclear whether individuals who show a good fasting glycaemic response to gliclazide also exhibit a good post-prandial glycaemic response to sitagliptin.

Clinical implications for future research

As our study has only investigated short term glycaemic response to two classes of diabetes therapy, it is unclear whether the lack of association in glucose-lowering response between sitagliptin and gliclazide is an isolated finding or whether it is representative of individual responses to all diabetes agents. In order to establish whether therapeutic response is specific to an individual, future

research would benefit from investigating glycaemic responses to multiple diabetes agents within an individual (achieved using a randomised control crossover design). As different diabetes agents work on different defective pathways in glucose homeostasis, all glycaemic outcomes should be measured at baseline and on-treatment, [4–7]), most importantly HbA1c response.

6.5 Conclusion

To conclude, we have shown that the fasting glycaemic response to stopping sulphonylurea therapy is associated with glycaemic response to re-starting SU therapy (gliclazide), but not with starting sitagliptin therapy. We have also shown that response to gliclazide therapy is not associated with the therapeutic response to sitagliptin (hypoglycaemic agent with differing mechanisms of action). These findings suggest that therapeutic response to glucose-lowering therapy is partly attributed to a biological characteristic of an individual and is specific to therapy.

6.6 References

1. Srivanichakorn W, Sriwijitkamol A, Kongchoo A, et al (2015) Withdrawal of sulfonylureas from patients with type 2 diabetes receiving long-term sulfonylurea and insulin combination therapy results in deterioration of glycemic control: a randomized controlled trial. *Diabetes Metab Syndr Obes* 8:137–145 . doi: 10.2147/DMSO.S78008
2. Landstedt-Hallin L, Arner P, Lins PE, et al (1999) The role of sulphonylurea in combination therapy assessed in a trial of sulphonylurea withdrawal. Scandinavian Insulin-Sulphonylurea Study Group Research Team. *Diabet Med* 16:827–834
3. Nybäck-Nakell A, Adamson U, Lins PE, Landstedt-Hallin L (2007) Glycaemic responsiveness to long-term insulin plus sulphonylurea therapy as assessed by sulphonylurea withdrawal. *Diabet Med* 24:1424–1429 . doi: 10.1111/j.1464-5491.2007.02286.x
4. Monami M, Adalsteinsson JE, Desideri CM, et al (2013) Fasting and post-prandial glucose and diabetic complication. A meta-analysis. *Nutr Metab Cardiovasc Dis* 23:591–598 . doi: 10.1016/j.numecd.2013.03.007
5. Standl E, Schnell O, Ceriello A (2011) Postprandial Hyperglycemia and Glycemic Variability: Should we care? *Diabetes Care* 34:S120–S127 . doi: 10.2337/dc11-s206
6. Kilpatrick ES, Rigby AS, Atkin SL (2008) A1C variability and the risk of microvascular complications in type 1 diabetes: data from the Diabetes Control and Complications Trial. *Diabetes Care* 31:2198–2202 . doi: 10.2337/dc08-0864
7. Takahashi S, Uchino H, Shimizu T, et al (2007) Comparison of glycated albumin (GA) and glycated hemoglobin (HbA1c) in type 2 diabetic patients: usefulness of GA for evaluation of short-term changes in glycemic control. *Endocr J* 54:139–144

Chapter 7

Chapter 7: Overall Conclusions from Thesis

7.1 Summary

In pursuit of identifying whether a stratified approach can be applied to T2D therapy, we investigated the potential mechanisms underpinning variation in therapeutic responses to sitagliptin and gliclazide.

Our findings demonstrate that variations in plasma drug levels and lifestyle factors (including adherence, weight and physical activity) do not substantially explain the variation in therapeutic response to sitagliptin and gliclazide therapy in patients with T2D. Our results do however show that worsening renal function is associated with higher plasma drug levels of sitagliptin, supporting current guidance to stratify patients receiving sitagliptin according to their degree of renal impairment.

We have also demonstrated that there may be a biological basis underpinning response to glucose-lowering medication, supported by the finding that a marked increase in fasting plasma glucose levels after stopping sulfonylurea therapy in the initial washout period correlated with a good glucose lowering response on restarting sulphonylurea therapy. Understanding the biological basis of this response and fundamentally, being able to identify individuals who exhibit this response could lend itself to the integration of a stratified approach to T2D pharmacotherapy.

Additionally, we found no association between the glycaemic responses to gliclazide versus sitagliptin in a given individual. This supports stratification, which will only be effective if poor response to one drug is associated with a good response to another drug with a different mechanism of action, further research needs to be done to see if there is any association in treatment response using different glucose-lowering therapies and/or combinations.

7.2 Strengths of our findings

To our knowledge, MASTERMIND is the first randomised controlled crossover trial to investigate factors influencing inter-individual variation to therapeutic response in T2D. The findings from this novel pilot study can help to direct further research in the field of precision diabetes.

A further strength of this thesis is the study from which the data were obtained used a randomised controlled crossover design, minimising bias and enabling individuals to act as their own control. Additionally, participants were assessed at multiple time points, allowing a more robust assessment of their glycaemic and clinical parameters.

Additionally, we assessed participants' adherence to medication using Medication Event Monitoring System (MEMS) caps, which are the gold standard. This enabled us to reliably assess whether non-response was truly due to biological non-response, or due to non-compliance.

7.3 Limitations of our findings

The main limitations of the trial were the length of the study (participants received only four weeks of each intervention) and the sample size.

The short study duration limited the assessment of the effect on body weight on glycaemia in individuals taking sitagliptin or gliclazide. It also meant we were unable to capture longer term variations in physical activity or change in weight reflective of individuals with T2D. Additionally, the short intervention periods impede the assessment of changes in HbA1c within our dataset, as HbA1c is reflective of glycaemic control over the preceding three months.

The sample size of 137 participants meant the study was insufficiently powered to detect small effects (particularly when performing subgroup analyses, as demonstrated when analysing our adherence data).

The above necessitates our findings to be validated in a larger data set with a longer study duration.

An additional limitation of the study was that only fasting blood glucose levels (without post prandial blood glucose levels) were obtained for both the start and end of each treatment period. This meant that we were limited to only calculating change in fasting glycaemia (and not post prandial glycaemia) when assessing therapeutic response over each intervention period.

Sitagliptin has little effect on fasting glycaemia over a short duration and mainly exerts its hypoglycaemic effects on post-prandial glucose, rendering us unable to

definitively assess its glycaemic effects. This in turn meant that we were unable to accurately examine the association between response to gliclazide (which predominantly affects fasting glucose levels) versus response to sitagliptin (which mainly influences post prandial glucose levels).

Further weaknesses include a limited assessment of baseline glycaemia following the MMTT (visit 2), where blood was sampled up to 2 hours compared to 4 hours during on-treatment MMTT visits. This had the potential to limit our adjustment for incremental $AUC_{0-240min}$ glucose response. However, this is unlikely to have been the case in this study, as the 2-hour baseline and 4-hour on-treatment MMTT incremental AUC glucose values highly correlated.

Lastly, study budget constraints limited analysis of plasma drug levels to less than half of participants that completed the study.

7.4 Clinical implications

The findings from the MASTERMIND pilot study have clinical implications for future precision medicine research in T2D. As variability in plasma drug levels and lifestyle factors (specifically adherence, weight and physical activity) were not associated with glucose-lowering response to sitagliptin and gliclazide, it is likely that complex measurement of these factors (for example, by using accelerometry and MEMS caps) in a controlled setting, is unlikely to be beneficial.

7.5 Future research

Avenues for future research include addressing the limitations outlined in this thesis. With respect to study design, we would benefit from increasing the duration of the intervention phases, as this would enable a more accurate representation of variations in body weight and physical activity in individuals with T2D on blood glucose lowering therapies, and the effect of these parameters on glycaemic response to therapy. It would also enable us to assess changes in HbA1c in response to glucose-lowering therapies, which is the gold standard means of assessing glycaemic control.

Double blinding the interventions would minimise detection and performance bias and avoid overestimation of the treatment effect. Aiming to recruit more participants would serve to increase the precision of our findings, especially when performing categorical analyses; e.g. determining whether individuals who are non-concordant with the treatment have differences in glycaemic response compared to concordant individuals. Expanding the geographic location from which subjects are recruited would also serve to optimise the study's external validity.

Despite this, recruiting and retaining larger participant cohorts is challenged by several economic and participant-related barriers [1]; discussion of which lies beyond the scope of this thesis.

In order to further address the question as to whether response to glucose-lowering agents is drug-specific or an inherent attribute of individual, future studies would benefit from comparing responses between agents that influence the same glycaemic markers (for example, GLP-1 receptor agonists and DPP-4 inhibitors mainly work by reducing post-prandial glucose). This would ensure a more accurate assessment of therapeutic response within individuals.

In addition to addressing the suitability of T2D for stratification, comparative trials could compare overall effects of treatment; including glucose lowering effects, side effect profile and patient preference. This would be particularly beneficial as there are limited head-to-head comparisons of newer glucose lowering therapies.

Finally, future research in precision diabetes may benefit from randomised controlled crossover trials that investigate therapeutic response to multiple diabetes agents (including sulphonylureas, GLP-1 receptor agonists, SGLT-2 inhibitors and thiazolidinediones), individually and in clinically appropriate combinations. Advantages of crossover trials include achieving adequate power with smaller numbers as participants serve as their own control. However, to go further and investigate variation in therapeutic response arising from interactions between participants and their treatment, future trials may benefit also from a repeated crossover design, where participants receives each medication more than once[2].

7.6 Final Remarks

Our findings demonstrate that measurable differences in drug absorption and lifestyle factors do not explain the substantial variation in therapeutic response to sitagliptin and gliclazide therapy. We have also shown that response to therapy is at least partly a reproducible and drug specific characteristic of an individual, suggesting that a stratified approach to T2D management may be feasible. Although future trials in stratified diabetes are necessary, our results suggest that detailed measurements of drug levels, adherence and physical activity are unlikely to be useful in this area.

7.7 References

1. Gul RB, Ali PA (2010) Clinical trials: the challenge of recruitment and retention of participants. *Journal of Clinical Nursing* 19:227–233 . doi: 10.1111/j.1365-2702.2009.03041.x
2. Lonergan M, Senn SJ, McNamee C, et al (2017) Defining drug response for stratified medicine. *Drug Discov Today* 22:173–179 . doi: 10.1016/j.drudis.2016.10.016